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(54) **Non-A non B hepatitis-specific antigen and its use in hepatitis diagnosis.**

(57) This invention relates to a DNA fragment comprising a base sequence encoding a non-A non-B hepatitis-specific antigen polypeptide, said base sequence being obtained using genetic engineering techniques from non-A non-B hepatitis virus RNA which is isolated directly from blood plasma from non-A non-B hepatitis patients, to an expression vector and a transformant for use in the expression of the DNA fragment, to a single strand DNA sequence for PCR primer, and to use of said polypeptide and said single strand DNA sequence in the detection of the non-A non-B hepatitis virus. The recombinant polypeptide and the single strand DNA sequence for PCR primer make it possible to detect the non-A non-B hepatitis virus with extremely high accuracy.

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The present invention relates to a novel DNA fragment encoding non-A non-B hepatitis-specific antigen polypeptide which is found at the time of infection or onset of the non-A non-B hepatitis.

It also relates to an expression vector containing said DNA fragment, to a transformant transformed with said expression vector and to an expressed polypeptide obtained by culturing said transformant.

5 It further relates to a single strand DNA sequence for PCR primer synthesized on the basis of a partial base sequence of said DNA fragment.

It also relates to the use of said expressed polypeptide and said single strand DNA sequence in detection of the non-A non-B hepatitis virus.

10 BACKGROUND OF THE INVENTION

Non-A non-B hepatitis is an infectious disease which is caused by a masked virus other than A and B hepatitis viruses, but it is not easy to identify the virus because an amount of the virus-specific antigens is very small in a patient's body as well as of anti-virus antibodies. Accordingly, diagnosis of non-A non-B hepatitis has been
15 made serologically by the well-known "diagnosis by exclusion" method wherein increase in the levels of alanine aminotransferase and aspartate aminotransferase is determined for a serum from a patient to make a diagnosis whether or not the hepatitis belongs to any of hepatitis A, hepatitis B, hepatitis D and other hepatitis symptoms due to the known hepatopathy-causing viruses such as CMV, EBV, etc, and if the result of diagnosis are not applicable to them, then a case is identified as non-A non-B hepatitis. It, however, is difficult to diagnose clinically as being non-A non-B hepatitis by such a method because there is no correlation between ALT value and
20 non-A non-B hepatitis. Also, the lack of trustworthy means for the diagnosis is a serious problem, whereby a secondary infection with the non-A non-B hepatitis virus which may be caused by transfusing blood, especially, from a non-A non-B hepatitis virus-carrying healthy carrier into a person can hardly be prevented. Therefore, it is considered that the non-A non-B hepatitis occupies more than 90% of hepatitis cases caused by blood
25 transfusion, with a total of about one million patients per one year.

In order to improve such situation and to raise a diagnostic accuracy of non-A non-B hepatitis, Alter's panel in which a standard serum is used has been developed by Alter *et al* at the NIH. Diagnostic materials which can pass the Alter's panel have been obtained by Arima *et al* [JIKKEN IGAKU (Japan), 7 (2), 196 - 201 (1989)] and by M. Houghton *et al* (WO 89/04669, PCT/JP90/500880) of Chiron Corp. almost simultaneously. Arima *et al*
30 *et al* have screened the sera from hepatitis patients using λ gt11 (a protein expression vector) which is derived from viral RNA from a non-A non-B hepatitis patient's serum. Also, Chiron Corp. have inoculated the patient's blood plasma into a chimpanzee to develop a chronic hepatitis, blood plasma being obtained from the diseased animal which possesses the anti-virus antibodies with high titer, and then have screened in the same way as Arima *et al*. Chiron Corp.'s group has also succeed in cloning almost the whole portion of the gene of a hepatitis
35 C virus (HCV, designated by Chiron Corp.) and developed a kit for diagnosis which comprises an antigen protein obtained by expressing a part of the HCV gene.

In spite of such an effort, however, factors of this disease, even their numbers, have not yet been elucidated to the full.

As described above, the two materials which can pass the Alter's panel has certainly lead to a new technique of diagnosis replaced by said "diagnosis by exclusion", but screening patient's sera separately with the
40 materials gives no results to be satisfied because both the materials from Arima *et al* and Chiron Corp. react with patient's sera in low positive ratios of about 60 to 80% and about 50 to 70%, respectively. In other words, in some cases, these materials would not react with sera from the patients who have been diagnosed clinically as non-A non-B hepatitis. A virus commonly have a function to cause mutation in their host cells for their survival, and thus the viral genes isolated from American patients by Chiron Corp. had been possibly mutated into various
45 forms acclimated to the chimpanzee as an infection intermediate.

Accordingly, a great demand has been directed to a large scale preparation of the reactive antigens which are capable of probing the non-A non-B hepatitis patients or carriers, therefore it will be necessary to construct effective cDNA clones through the isolation and purification of variously mutated viral RNA from many non-A
50 non-B hepatitis patients.

In addition, in the case of sera which have failed in a trustworthy diagnosis using an antibody detection system, or of sera which are collected immediately after infection and in which antibody titers do not yet raise, a gene amplification method (PCR method) may be useful for the confirmation of the disease because it can detect a trace amount of viral genes. Also, it is possible to clone the genes efficiently by the PCR method. However,
55 since the PCR method is carried out using primers which are synthesized from a known gene sequence, it is not always possible to detect a gene of the non-A non-B hepatitis virus in a patient's fluid using a primer(s) which can be constructed on the basis of the HCV gene sequences determined by Chiron Corp., if a difference in mutation between said HCV gene of Chiron Corp. and said patient-carried viral gene is significant.

In consequence, to detect efficiently infection with the non-A non-B hepatitis virus, it is necessary to prepare at least one primer capable of detecting the viral gene with a high specificity. Such a purpose may be accomplished by isolating a great number of cDNA clones, synthesizing primers from relatively preserved regions among their gene sequences, and subjecting the primers obtained to screening through the PCR method.

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SUMMARY OF THE INVENTION

This invention provides a novel DNA fragment which encodes a non-A non-B hepatitis-specific antigen polypeptide originated from a non-structural or structural protein of the non-A non-B hepatitis virus, the polypeptide being formed at the time of the infection or onset of the non-A non-B hepatitis.

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This invention also provides an expression vector containing the DNA fragment, a transformant transformed with the expression vector, an expressed polypeptide obtained by culturing the transformant, and a process for its production.

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This invention further provides a primer for use in the detection of non-A non-B type hepatitis virus genes.

This invention further yet provides use of the expressed polypeptide or single strand DNA primer in detection of the non-A non-B hepatitis virus, and a method for the detection of non-A non-B type hepatitis virus genes and anti-non-A non-B type hepatitis virus antibodies.

BRIEF DESCRIPTION OF THE DRAWINGS

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Fig. 1 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C11-7 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 1.

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Fig. 2 shows a nucleotide sequence of non-A non-B hepatitis specific-cDNA which is encoded in a clone C10-11 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 2.

Fig. 3 shows a nucleotide sequence of non-A non-B hepatitis specific-cDNA which is encoded in a clone C10-13 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 3.

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Fig. 4 shows a nucleotide sequence of non-A non-B hepatitis specific-cDNA which is encoded in a clone C10-14 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 4.

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Figs. 5 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-15 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 5.

Fig. 6 shows a nucleotide sequence of non-A non-B hepatitis specific-cDNA which is encoded in a clone C10-16 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 6.

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Fig. 7 shows a nucleotide sequence of non-A non-B hepatitis specific-cDNA which is encoded in a clone C10-17 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 7.

Figs. 8 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-18 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 8.

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Fig. 9 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-19 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 9.

Figs. 10 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-21 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 10.

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Fig. 11 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-22 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 11.

Fig. 12 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-23 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 12.

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Fig. 13 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-35 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 13.

ID NO. 13.

Fig. 14 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C11-C21 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 14.

Fig. 15 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-E12 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 15.

Fig. 16 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-E13 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 16.

Fig. 17 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-E24 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 17.

Fig. 18 shows a nucleotide sequence of non-A non-B hepatitis-specific cDNA which is encoded in a clone C10-E15 and an amino acid sequence deduced from the sequence. These sequences are equivalent to SEQ ID NO. 18.

Fig. 19 shows a flow sheet for the construction of an expression plasmid Trp-TrpE-C11-7.

Fig. 20 shows a flow sheet for the construction of an expression plasmid Trp-TrpE-C11-C21.

Fig. 21 is a photograph showing the results of western blotting analysis of an expressed product, TrpE-C11-7, with serum from a normal person or non-A non-B hepatitis patient, wherein the antigens used are a purified antigen in A, an extract of expressed cells in B, and an extract of non-expressed cells in C.

Fig. 22 is a photograph showing the results of western blotting of an expressed product, TrpE-C11-C21, with sera (A, B) from two normal persons or non-A non-B hepatitis patients.

Fig. 23 is a graphical representation of the ELISA-determined positive numbers in Table 4.

DETAILED DESCRIPTION OF THE INVENTION

Many aspects and advantages of the present invention will be made apparent to those skilled in the art by the following detailed description about preferred embodiments of the invention.

The present invention provides a specified DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural or structural protein of the non-A non-B type hepatitis virus.

In preparation of the DNA fragment of the present invention, it is characterized that variously mutated genes of pathogenic viruses were directly collected from fresh blood plasma pools of a number of non-A non-B hepatitis patients. More particularly, the preparation comprises the steps in which total RNA molecules including non-A non-B hepatitis virus RNA are isolated from the blood plasma pool, cDNAs are synthesized based on the isolated RNA molecules by the well-known random primer method, and then the cDNAs obtained are incorporated into λ phage to prepare a cDNA library. The cDNA library is subsequently immunoscreened using sera from a non-A non-B hepatitis patient to obtain the DNA fragments of interest. Thereafter, using the resulting DNA fragments as probe, cDNA libraries obtained from the blood plasma from several chronic non-A non-B hepatitis patients were subjected to hybridization assay in order to isolate a cDNA which has different homology from the known counterparts and which is specific for the non-A non-B hepatitis patient.

Such a process makes it possible to provide the viral antigens which are markedly useful for the diagnosis of non-A non-B hepatitis patients carrying the variously mutated viruses and for the improvement of detection accuracy of the hepatitis viruses contained in blood for transfusion which was collected from many latent carriers carrying non-A non-B type hepatitis viruses.

The following describes the present invention in detail with regard to the preparation of cDNA library, isolation and sequencing of DNA fragments, expression and isolation of polypeptides, and their application to diagnosis of non-A non-B hepatitis using enzyme-linked immunosorbent assay (ELISA) or PCR method.

Preparation of cDNA library

Firstly, cell debris is removed from each of freshly collected blood plasma samples of several non-A non-B hepatitis patients by centrifugation and the resulting supernatant is again subjected to centrifugation at a higher speed of rotation to obtain a pellet. The pellet is subjected to an equilibrium density gradient centrifugation using cesium trifluoroacetate to isolate total RNA as a precipitate, and the total RNA is purified by phenol/chloroform extraction and ethanol precipitation.

By the method of Gubler and Hoffman using random primers, cDNA is synthesized from the above RNA

fraction. The cDNA is methylated by treating it with a DNA methylase (for example, *EcoRI* methylase), connected with a DNA linker (for example, *EcoRI* linker) or DNA adapter (for example, *EcoRI* adapter), and then cloned into a cloning vector such as λ phage (for example, λ gt10 or λ gt11) to prepare a cDNA library.

5 Isolation and sequencing of DNA fragments

Next, *Escherichia coli* is infected with the λ phage cDNA library and cultured on an agar plate to form plaques. These plaques are transferred on a nitrocellulose filter, and subjected to blocking followed by immunoscreeing using a non-A non-B hepatitis serum in order to detect positive clones. Alternatively, to improve efficiency of the screening, each positive clone obtained is cloned into a cloning vector such as plasmid and a ^{32}P -labeled DNA probe is prepared by random primer technique, and then positive plaques are detected from the aforementioned cDNA library using the probe.

Eighteen clones in total were obtained by the above procedure and designated as C11-7, C10-11, C10-13, C10-14, C10-15, C10-16, C10-17, C10-18, C10-19, C10-21, C10-22, C10-23, C10-35, C11-C12, C10-E12, C10-E13, C10-E24 and C10-E15.

A cDNA sample is obtained from λ phage DNA of each 18 clones in a traditional manner and digested with appropriate restriction enzymes such as *EcoRI* and *BamHI*. Each cDNA fragment obtained is purified by agarose gel electrophoresis, incorporated into a sequencing vector (M13 phage), and then subjected to the dideoxy chain termination method [Sanger *et al.*, *Proc. Natl. Acad. Sci.*, USA, 74, 5463 (1977)] in order to determine a base sequence of each cDNA fragment.

Nucleotide sequences of these clones and deduced amino acid sequences are shown in Figs. 1 to 18 and in a Sequence Listing which will be described later as SEQ ID NOs. 1 to 18. That is, the SEQ ID NOs. 1 to 18 respectively represent the nucleotide and deduced amino acid sequences determined from clones C11-7, C10-11, C10-13, C10-14, C10-15, C10-16, C10-17, C10-18, C10-19, C10-21, C10-22, C10-23, C10-35, C11-C12, C10-E12, C10-E13, C10-E24 and C10-E15. Also, the base pair (BP) number of their DNA fragments is 763 BP, 615 BP, 771 BP, 630 BP, 1426 BP, 855 BP, 315 BP, 911 BP, 489 BP, 1076 BP, 284 BP, 641 BP, 432 BP, 369 BP, 932 BP, 559 BP, 276 BP and 742BP, respectively.

All the 18 clones contained a continuous open reading frame but with no termination codon.

Analysis of genomic RNA has revealed that hepatitis C virus (HCV) is a class of virus similar to the genus *Flavivirus* such as Japanese encephalitis virus [*Protein, Nucleic Acid and Enzyme* (Japan), 35 (12), 2117 - 2127 (1990)]. From the comparison of homology between the reported gene and polypeptide of *Flavivirus* and those of the present invention, it was found that clones C11-C12, C10-E12, C10-E13, C10-E24 and C10-E15 encode a structural protein of the non-A non-B type hepatitis virus. More particularly, clone C11-C12 is a gene which encodes the core of non-A non-B hepatitis virus, and clones C10-E12, C10-E13, C10-E24 and C10-E15 are genes encoding a region from the latter half of the virus core to the env or a region downstream from the env. Other clones were found to be genes encoding non-structural proteins of the virus.

The nucleotide sequences of the above 18 clones and the amino acid sequences translated along the open reading frames showed homologies with those of hepatitis C virus (HCV) reported by Houghton *et al.* (EP-A-318,216, 1988). In other words, clones C11-7, C10-16, C10-17, C10-18, C10-19, C10-21, C10-22 and C10-23 showed relatively high homologies with HCV: 80 to 82% homology at nucleic acid level and 91 to 94% at amino acid level. In addition, these clones showed more higher homologies with the sequence J1 reported by Miyamura *et al.* (*Nuc. Aci. Res.*, 17, 10367 - 10372, 1989): 85 to 95% homology at nucleic acid level and 87 to 100% at amino acid level. These clones were classified as group 1 because of high homology in their overlapped portion. On the contrary, clones C10-11, C10-13, C10-14, C10-15 and C10-35 showed low homologies when compared to the nucleotide and amino acid sequences of HCV and J1, i.e., 69 to 70% homology at nucleic acid level and 75 to 80% at amino acid level. they were therefore classified as group 2.

In addition, when the 369 BP nucleotide and deduced 123-amino acid sequences, indicated as SEQ ID NO. 14, for the C11-C21 clone encoding a structural protein of the virus were compared with the portions overlapped with HCV reported by Houghton *et al.* (WO 90/11089), a nucleic acid homology of 81.8% and an amino acid homology of 87% were found. Also, when compared with HCV clones, HC-J1 and HC-J4, obtained from a Japanese patient (Okamoto *et al.*; *Japan J. Exp. Med.*, 60, 3, p. 167 - 177, 1990), homologies of 82.1% and 82.7% at nucleic acid level and 87.8% and 89.4% at amino acid level were shown. Since the same regions among the reported three clones (HCV by Houghton *et al.* and HC-J1 and HC-J4 by Okamoto *et al.*) have high homologies of 92.1 to 97.6% at nucleic acid level and 95.5 to 96.7% at amino acid level, it has been found that the clone C11-C21 obtained by the present inventors has a certain distance from the reported clones in terms of homology and therefore is a different group of viral gene therefrom. The remaining 4 clones, C10-E12, C10-E13 and C10-E15, showed homologies of 83 to 93% at nucleic acid level and 82 to 95% at amino acid level when compared with the HCV, HC-J1 and HC-J4, while C10-E24 showed around 63% of homology at nucleic

acid level and around 60% of homology at amino acid level.

However, no homology was found either at nucleic acid level or amino acid level, when the DNA fragments of the present invention were compared with any DNA fragment encoding non-A non-B hepatitis antigens which have been disclosed in Japanese patent Application Laying-Open (KOKAI) Nos. 89/2576 and 89/124387.

5 Consequently, the clones C10-11, C10-13, C10-14, C10-15, C10-35, C11-C12, and C10-E24 have low homologies with the reported clones both at nucleic acid and amino acid levels. Other clones are also distinguishable from the reported clones.

Therefore, the present invention provides a DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide, said polypeptide consisting of the whole or a part of the amino acid sequence which is encoded along the open reading frame and represented by any one of the SEQ ID NOs. 1 to 18.

Naturally, the base sequences according to the present invention include any other base sequence which comprises other codons corresponding to each amino acid.

Among the aforementioned clones, C11-7, C10-11, C10-13, C10-14, C10-15, C10-16, C10-17, C10-18 and C10-19 were transformed into *E. coli* HB101 strain and deposited on July 6, 1990 with Fermentation Research Institute, Agency of Industrial Science and Technology, 1-3 Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305, Japan, respectively as *E. coli* HB101/C11-7 (Accession Number: FERM P-11589), *E. coli* HB101/C10-11 (FERM P-11581), *E. coli* HB101/C10-13 (FERM P-11582), *E. coli* HB101/C10-14 (FERM P-11583), *E. coli* HB101/C10-15 (FERM P-11584), *E. coli* HB101/C10-16 (FERM P-11585), *E. coli* HB101/C10-17 (FERM P-11586), *E. coli* HB101/C10-18 (FERM P-11587) and *E. coli* HB101/C10-19 (FERM P-11588). These depositions were subsequently converted on June 13, 1991 to an international deposition under Budapest Treaty by the same depositary institution as an international depositary authority set forth in Budapest Treaty to be given the following new Accession Numbers:

25	<u><i>E. coli</i> HB101</u>	<u>Accession No. (FERM BP-)</u>
	Clone C11-7	3442
	Clone C10-11	3434
30	Clone C10-13	3435
	Clone C10-14	3436
35	Clone C10-15	3437
	Clone C10-16	3438
	Clone C10-17	3439
40	Clone C10-18	3440
	Clone C10-19	3441

45 Also, clones C11-C21, C10-E12, C10-E13, C10-E24 and C10-E15 were transformed into *E. coli* JM109 strain and deposited on December 11, 1990 with Fermentation Research Institute, Agency of Industrial Science and Technology, the same address, respectively under Accession Numbers FERM P-11892, FERM P-11894, FERM P-11895, FERM P-11896 and FERM P-11897. These depositions were also subsequently converted on June 17, 1991 for clone C11-C12 and on June 13, 1991 for other clones to an international deposition under Budapest Treaty in the same way. The following new Accession Numbers were given:

E. coli JM109 Accession No. (FERM BP-)

5	Clone C11-C21	3450
	Clone C10-E12	3444
	Clone C10-E13	3445
10	Clone C10-E24	3446
	Clone C10-E15	3447

As described in the foregoing, the DNA fragments according to the present invention are different from any other prior DNA fragment. Though non-A non-B hepatitis virus is generally divided into two classes, namely groups 1 and 2, on the basis of the comparison of homology between the clones encoding a non-structural reagent of the hepatitis virus, there is a possibility of existing an intermediate group or even a third group because the virus is very susceptible to mutation in its host cells. It may be accordingly difficult to correctly diagnose all the non-A non-B hepatitis patients using an antigen protein prepared from only one kind of DNA fragment. In order to overcome such a problem and to improve an efficiency of the diagnosis, it is necessary to establish such a useful process for the preparation of DNA that a number of effective clones can easily be obtained, and to use several types of clones in combination in diagnosis

Expression of non-A non-B hepatitis specific antigen polypeptide

The present invention also provides an expression vector which is constructed by introducing the above-mentioned DNA fragment into a cloning site downstream of a promoter gene in a vector.

Any conventional vector may be used such as plasmid, phage or the like. An expression vector may be constructed by the well-known techniques in the art. The following describes some processes for constructing the expression vectors of the invention.

Construction of expression plasmid Trp-TrpE-C11-7:

A flow sheet for the construction of the expression plasmid Trp-TrpE-C11-7 is shown in Fig. 19.

Firstly, a plasmid pUC-C11-7 DNA obtained by incorporating the clone C11-7 into pUC119 is digested with restriction enzymes *Bam*HI and *Sal*I, and the resulting *Bam*HI - *Sal*I fragment is isolated by agarose gel electrophoresis and then purified by a glass powder technique. Separately from this, an expression vector Trp-TrpE DNA is digested with *Bam*HI and *Sal*I, treated with a bacterial alkaline phosphatase (BAP), and then extracted with phenol. The aqueous layer obtained is subsequently subjected to ethanol precipitation to obtain a treated vector DNA. By connecting the vector DNA with the aforementioned C11-7 DNA fragment in the presence of T4 DNA ligase, the expression plasmid Trp-TrpE-C11-7 is obtained in which the DNA encoding the non-A non-B hepatitis-specific antigen is located downstream of a promoter so that transcription of the DNA can be controlled by the promoter.

Construction of expression plasmid Trp-TrpE-C11-C21:

A flow sheet for the construction of the expression plasmid Trp-TrpE-C11-C21 is shown in Fig. 20.

Firstly, a DNA fragment containing a stop codon in its 3' terminal is prepared from a plasmid pUC-C11-C21 DNA which is obtained by incorporating the C11-C21 clone into pUC119, by a gene amplification method (PCR) using two primers (5'-TTACGAATTCATGGGCACGAATCCT-3' and 5'-TTAATCGATGACCTTACCCA-CATTGCG-3'). By ligating the thus-prepared DNA fragment with pUC118 which is predigested with *Sma*I, a plasmid pUC118-C11-C21-*Sma*I is obtained. This plasmid is then digested with *Eco*RI and *Bam*HI, and the resulting DNA fragment is isolated by agarose gel electrophoresis and then purified by glass powder technique. Separately from this, an expression vector Trp-TrpE DNA (Japanese Patent Application No. 90/180889) is digested with *Bam*HI and *Eco*RI, treated with a bacterial alkaline phosphatase (BAP), and then extracted with phenol. The aqueous layer obtained is subsequently subjected to ethanol precipitation to obtain a treated vector DNA. By connecting the vector DNA with the aforementioned C11-C21 DNA fragment by the action of T4 DNA ligase in a ligation buffer solution, the expression plasmid Trp-TrpE-C11-C21 is obtained in which the DNA-en-

coded polypeptide from a structural protein of the non-A non-B hepatitis virus is located downstream of a promoter so that transcription of the DNA can be controlled by the promoter.

Other clones can also be made into corresponding expression plasmids by treating each clone with appropriate restriction enzymes and introducing the treated fragment into an expression vector.

5 When a procaryote is used as the host cell, a promoter eligible for use in the present invention may be selected from promoters originated from *E. coli*, phage and the like, such as tryptophan synthase operon (*trp*), lactose operon (*lac*), λ phage P_L , λ phage P_R and the like. When an eucaryote such as yeast is used as the host cell, promoters for 3-phosphoglycerate kinase and other glycolysis-related enzymes (Holland *et al*; *Biochemistry*, 17: 4900, 1978) may be useful. Though not always required, a transcription termination factor

10 may preferably be located in the expression vector. The vector may further contain a marker sequence, such as an ampicillin or a tetracycline resistance gene, which makes it possible to effect a phenotype selection in transformed cells.

The present invention also provides a transformant which is obtained by introducing the expression vector of the invention into a host cell. Microorganisms used commonly in this field, such as *E. coli*, *B. subtilis*, a yeast

15 strain and the like, may be used as a host cell. Transformation may be effected by any usually used means for the incorporation of an expression vector into host cells. When a bacterium (for example, *E. coli*) is used as host cell, a direct incorporation technique with the use of calcium chloride (Mandel, M. and Higa, A; *J. Mol. Bio.*, 53, 159 - 162, 1970) may be employed.

In addition, the polypeptide of the present invention may be produced by inoculating and culturing a suitable

20 host cell carrying the expression vector in an appropriate medium such as ampicillin-containing 2YT medium and then propagating expression cells by subculturing them in an ampicillin-containing phosphate medium.

Production and purification of recombinant non-A non-B hepatitis-specific antigen polypeptide

25 The present invention also provides a process for producing a non-A non-B hepatitis-specific antigen polypeptide, which comprises the following steps of:

constructing a replicable expression vector which can express the aforementioned DNA fragment of the present invention in an appropriate host cell;

obtaining a transformant by incorporating said expression vector into the host cell;

30 producing a recombinant polypeptide by culturing said transformant under such conditions that said DNA fragment can be expressed; and

recovering said recombinant polypeptide.

The crude polypeptide product from host cells may be purified by disintegration of the host cells, for example by ultrasonic disintegration, subjecting the disintegrated cells to centrifugation to obtain an insoluble fraction

35 containing a fused polypeptide between TrpE as signal peptide and a polypeptide encoded by cDNA synthesized from a non-A non-B hepatitis virus RNA, extracting the fused polypeptide in a soluble form with a urea-containing buffer, and then purifying the extracted polypeptide by subjecting it to an ion exchange column chromatography (S-Sepharose, for example).

Accordingly, the present invention also provides a recombinant non-A non-B hepatitis-specific antigen

40 polypeptide obtained by such a expression process, said polypeptide consisting of the whole or a part of the amino acid sequence represented by any one of the SEQ ID NOs. 1 to 18.

The term "recombinant non-A non-B hepatitis-specific antigen polypeptide" as used herein is intended to include a polypeptide itself which is obtained by expressing in a vector a DNA fragment encoding a non-A non-B hepatitis-specific antigen polypeptide, and a fused polypeptide obtained by fusing said polypeptide with other

45 peptide such as a signal peptide.

Application to diagnosis of non-A non-B hepatitis

The expressed polypeptide of the present invention was subjected to SDS-polyacrylamide gel

50 electrophoresis and then allowed to perform antigen-antibody reaction with each two serum samples from normal persons or non-A non-B hepatitis patients by means of western blotting, whereby this polypeptide reacted strongly with only the patient's sera as shown in Figs. 21 and 22. It was confirmed therefore that the expressed polypeptide functions as a non-A non-B hepatitis-specific antigen.

Accordingly, the present invention also provides a method for immunological detection to detect an anti-

55 body directed against the non-A non-B hepatitis virus antigen, which comprises the following steps of:

incubating a sample possibly containing an anti-non-A non-B hepatitis virus antibody together with at least one recombinant non-A non-B hepatitis-specific antigen polypeptide of the present invention under such conditions that the antigen is capable of reacting immunologically with the antibody; and

detecting an antigen-antibody complex.

Diagnostic effects (positiveness) of the expressed polypeptide TrpE-C11-C21 obtained by expressing the expression plasmid Trp-TrpE-C11-C21, another expressed polypeptide TrpE-C11-7 obtained by expressing the corresponding expression plasmid Trp-TrpE-C11-7, and an assay kit of Chiron Corp. (ORTHO HCV Ab ELISA kit) were examined by the conventional enzyme immunoassay through the reaction of the above expressed antigens with a serum sample from a patient who has been diagnosed clinically as being non-A non-B hepatitis. As the results, positiveness of the kit of Chiron Corp. was found to be 69.7% (23/33 cases) while the TrpE-C11-7 which belongs to group 1 showed a positiveness of 78.8% (26/33 cases). In the case of the expressed polypeptide TrpE-C11-C21, it showed a positiveness of 84.8% (28/33 cases) which is higher than the case of the Chiron's kit. When the expressed polypeptide TrpE-C11-7 as a member of group 1 and the TrpE-C11-C21 as a member of group 2 were used in combination, the positiveness increased to 93.9% (30/31 cases; see Table 1 and Fig. 23).

Therefore, according to an embodiment of the present invention, there is provided a combination of the group 1 and group 2-relating expressed polypeptides as a hepatitis-specific antigen polypeptide for use in the immunological detection.

The present invention further provides a method for gene amplification which comprises amplifying a non-A non-B hepatitis virus gene using sense and/or antisense sequence synthesized on the basis of the DNA sequences of the present invention.

As the synthetic base sequence for PCR primer, the following single strand DNA sequences may be employed:

5'-GGATACACCGGTGACTTTGA-3'(sense, SEQ ID NO. 19);
5'-TGCATGCACGTGGCGATGTA-3'(antisense, SEQ ID NO. 20);
5'-GATGCCCACTTCCTCTCCCA-3'(sense, SEQ ID NO. 21); and
5'-GTCAGGGTAACCTCGTTGGT-3'(antisense, SEQ ID NO. 22).

said sequences being sense or antisense of the partial base sequence represented by the SEQ ID NO. 5 for the former two primers and by the SEQ ID NOs. 2, 4, 5 or 13 for the latter two primers. These specified primers are also within a scope of the invention.

The single strand DNA sequences may be synthesized by the usual methods such as phosphorous acid method, phosphotriester method, solid phase method and the like, though the use of a DNA synthesizer is most convenient.

When used as a PCR primer, the above single strand DNA sequences show higher specificity for the group 2 virus genes than for the group 1 virus genes (see Tables 2 and 3).

Therefore, the present invention also provides a method for detecting the genes from the non-A non-B type hepatitis virus in a fluid sample such as serum, which comprises the following steps of:

isolating RNA from the sample,
synthesizing cDNA by treating the obtained RNA with a reverse transcriptase,
subjecting the obtained cDNA to polymerase chain reaction using at least one the above-mentioned primer,
detecting an amplified non-A non-B type hepatitis virus gene.

The present invention further provides use of the expressed polypeptides or single strand DNA sequences for PCR primer of the present invention in the detection of the non-A non-B hepatitis virus.

The following examples are given to further illustrate the present invention in detail, but it is not intended to limit the invention thereby.

Example 1

Preparation of cDNA library from blood plasma of non-A non-B hepatitis patient

A cDNA library was prepared using λ gt10 and λ gt11 phages after preparing an RNA fraction in the following manner from fresh blood plasma pools obtained from several Japanese patients of chronic stage non-A non-B hepatitis.

Five liter of blood plasma was diluted with the equal volume of 50 mM Tris-HCl (pH 8.0) containing 1 mM EDTA, cell debris in the diluted sample was removed by centrifugation at 3,500 g for 20 minutes and then the resulting supernatant was again subjected to centrifugation at 45,000 rpm (about 100,000 g) for 4 hours at a temperature of 4°C to obtain pellet. The pellet was dissolved, according to the conventional procedure, in 6M guanidium thiocyanate as a protein denaturing agent, layered over a solution of cesium trifluoroacetate, and then subjected to centrifugation using Beckman SW50 rotor at 33,000 rpm for 18 hours at a temperature of 20°C. The resulting pellet was dissolved in 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA and extracted twice

with a solvent system of 1:1 phenol:chloroform, after which the organic layer was mixed with 1/10 volume of 5 M NaCl and 2.5 volumes of ethanol. After standing the mixture for 2 hours at -20°C, it was centrifuged at 15,000 g for 20 minutes and the pellet was then dissolved in diethylpyrocarbonate-treated water to use as an RNA sample.

In accordance with the method of Gubler and Hoffman, cDNA was synthesized from the thus obtained RNA sample by means of random primer technique using a commercially available kit (from Amersham or BRL). The cDNA was subsequently treated with *EcoRI* methylase, ligated with an *EcoRI* linker or an *EcoRI* adapter and then cloned into the *EcoRI* site of λ gt10 and λ gt 11 phages. The cDNA library thus prepared contained 10^6 to 10^7 PFU of recombinant phages in average.

Example 2

Isolation of non-A non-B hepatitis-specific cDNA

An attempt was made to isolate cDNA specific for non-A non-B hepatitis from the cDNA library prepared in Example 1, by immunoscreening and hybridization assay.

Firstly, immunoscreening of λ gt11 library was carried out using two serum samples from non-A non-B hepatitis patients which are negative for HBc and HBs antibodies and which contain antibodies specific for the hepatitis-causing virus. Immunoscreening was performed in the usual way by examining specific reaction of a β -galactosidase-fused recombinant peptide with a serum sample of non-A non-B hepatitis (to be referred to as "NANBH" herein after) patient.

Cells of *E. coli* Y1090 strain were mixed with λ gt11 cDNA library at a predetermined ratio, plated on an agar medium at an appropriate density, and then incubated at 43°C for 3 hours to form plaques. Next, the agar plate was covered with a Hybond-C nitrocellulose filter which has been soaked with 10 mM IPTG and the filter-covered plate was incubated again at 37°C for 3 hours to induce expression. Subsequently, the nitrocellulose filter was subjected to blocking using 3% gelatin solution, reacted with a serum sample of NANBH patient overnight at 4°C, and then, after washing, reacted with a peroxidase-labeled anti-human IgG (goat antibody). A positive signal was found when the resulting filter was reacted with a mixture of diaminobenzidine and H_2O_2 . This clone, C11-7, did not react with HBc and HBs antibodies.

Next, in order to improve efficiency of the screening, the clone C11-7 was re-cloned into pUC119 and made into a probe by random primer method. Using the probe, λ gt10 cDNA library was screened by means of hybridization assay. Screening was carried out according to the conventional method by plating 5×10^4 PFU of recombinant phages with *E. coli* C-600 hfl(-) on an L-plate (150 mm dish). When plaques appeared after overnight incubation of the plate at 37°C, the plate was stored at 4°C for 1 hour and thereafter the plate was covered with a Hybond-N filter for a period of 30 seconds. The resulting filter was superposed for 1 minute on a filter prewetted with a denaturing solution (0.5 M NaOH and 1.5 M NaCl), soaked for 5 minutes in a neutralizing solution (0.5 M Tris-HCl pH 7.0 and 1.5 M NaCl), washed with 2 x SSC, and then dried. The filter was subjected to UV-crosslinking by exposing it to UV rays (304 nm) for 2 minutes. Thereafter, as described below, the resulting filter was subjected to screening by hybridization assay using a ^{32}P -labeled DNA probe which has been prepared by random primer method from the C11-7 clone obtained by immunoscreening with a serum from NANBH patient.

The filter was incubated overnight at 65°C in 1 x SSC, washed twice with 1 x SSC at 65°C (10 minutes for each) and then subjected to autoradiography at -70°C for the detection of positive plaques. Each positive plaque was transferred into SM buffer and used as a phage stock. Clones obtained were used as marker probe to carry out a series of screening. As the results, 13 clones in total were isolated and designated as C10-11, C10-13, C10-14, C10-15, C10-16, C10-17, C10-18, C10-19, C10-21, C10-22, C10-23 and C10-35.

Example 3

Selection isolation of group 2 non-A non-B hepatitis-specific cDNA

A blood plasma sample which can react only with C10-14 clone was obtained by subjecting fresh blood plasma of a Japanese patient in a chronic phase of the non-A non-B hepatitis to an ELISA-based screening system, using expressed products of the group 1 cDNA clone C11-7 and the group 2 cDNA clone C10-14 isolated in Examples 1 and 2. This blood plasma sample was subjected to a gene amplification method (PCR method) using well preserved primers of group 1 and those of group 2. PCR method was carried out using Gene Amp™ (DNA Amplification Reagent Kit, Perkin Elmer Cetus) under conditions of: DNA denaturation, 95°C for 1.5 minutes; annealing, 55°C for 2 minutes; and DNA synthesis, 70°C for 3 minutes. Blood plasma samples in

which gene amplification was found only with the use of the group 2 primers under these conditions were pooled for further use. An RNA fraction was prepared from one liter of this fresh blood plasma sample in the same manner as in Example 1, and a cDNA library (referred to as "cDNA library A" hereinafter) was constructed using λ gt10 and λ gt11 phages. The cDNA library A contained 10^6 to 10^7 PFU of recombinant phages in average.

On the other hand, a cDNA library B was constructed using λ gt10 phage from five liters of fresh blood plasma samples which have been collected as starting material from several patients of non-A non-B hepatitis and have not been subjected to the ELISA/PCR method, in the same manner as described above. The cDNA library B also contained 10^6 to 10^7 PFU of recombinant phages in average.

Cloning of non-A non-B hepatitis-specific cDNA from cDNA library A was carried out by immunoscreening in the same manner as in Example 2, and a positive plaque (clone C11-C21) was obtained. The clone C11-C21 showed no positive reaction with HBc and HBs antibodies.

In order to improve efficiency of the screening, the thus obtained clone C11-C21 was re-cloned into pUC119, digested with restriction enzymes, and then made into a 32 P-labeled probe by random primer labeling method in the same manner as in Example 2. Using the probe obtained, the cDNA library B was screened by hybridization assay. After a series of the screening efforts, 4 clones were isolated and named C10-E12, C10-E13, C10-E24 and C10-E15.

Example 4

Sequencing of non-A non-B hepatitis-specific cDNA

E. coli cells were infected with the λ gt11 or λ gt10 phage of each of the 18 clones obtained in Examples 2 and 3 to recover respective phage in a large quantity. DNA was extracted from the phage by the conventional alkali method, digested with a restriction enzyme *EcoRI*, *BamHI* or *KpnI*, and the resulting DNA fragments were purified by agarose gel electrophoresis. Separately from this, sequencing vectors mp18 and mp19 of M13 phage (Messing, J.; *Methods in Enzymology*, 101, 20 - 78) or pUC118 and pUC119 (Vieira, J. and Messing, J.; *Methods in Enzymology*, 153, 3 - 11) were digested with a restriction enzyme *EcoRI*, *BamHI* or *KpnI* to obtain linear vector fragments. The cDNA fragment and the vector DNA were linked together using T4 ligase in a buffer solution, and the resulting reaction product was incorporated into *E. coli* HB101 or JM109 strain by transformation or transfection. Resulting *E. coli* cells were cultured and DNA was recovered by alkali method. Nucleotide sequence of the DNA obtained was determined according to the dideoxy chain termination method of Sanger *et al.*

The nucleotide sequences of clones C10-11, C10-13, C10-14, C10-15, C10-16, C10-17, C10-18, C10-19, C10-21, C10-22, C10-23, C10-35, C10-C21, C10-E12, C10-E13, C10-E24 and C10-E15 and the amino acid sequences deduced from these nucleotide sequences are shown in a sequence table as SEQ ID NOs. 1 to 18 and also in Figs. 1 to 18.

On the basis of the comparison of homologies among these sequences and the nucleotide and deduced amino acid sequences disclosed by Houghton *et al.* (WO89/04669, PCT/JP90/500880) and Miyamura *et al.* (*Nuc. Ac. Res.*, 17, 10367-10372(1989)), clones C11-7, C10-17, C10-18, C10-19, C10-21, C10-22 and C10-23 obtained in Example 2 were classified as group 1 clone as defined hereabove while clones C10-11, C10-13, C10-14, C10-15 and C10-35 were classified as group 2 clones. Every of these 13 clones encoded non-structural protein of the non-A non-B type hepatitis virus. Moreover, clone C10-C21 in Example 3 was classified as group 2 from the comparison of homology with the sequences described by Houghton *et al.* (WO90/11089) and Okamoto *et al.* (*Japan J. Exp. Med.*, 60, 3, pp.167-177 (1990)), but classification of the clones C10-E12, C10-E13, C10-E24 and C10-E15 in Example 3 is not still clear. However, it was found that these 5 clones encode the structural protein of non-A non-B hepatitis virus from the comparison of homology with the reported genome of *Flavivirus* (*Protein, Nucleic Acid and Enzyme* (Japan), 35 (12), 2117-2127 (1990)).

Example 5

Expression and purification of polypeptide encoded by non-A non-B type hepatitis virus cDNA

(i) Construction of expression plasmid Trp-TrpE-C11-7:

One of the clones isolated, C11-7, was expressed as a fused polypeptide with TrpE in *E. coli* under the control of Trp promoter (see Fig. 19).

Firstly, 1 μ g of a plasmid pUC-C11-7 DNA which has been obtained by incorporating the C11-7 clone into pUC119 was digested by incubating it at 37°C for 1 hour in 20 μ l of a restriction enzyme reaction solution [150

mM NaCl, 6 mM Tris-HCl (pH 7.9), 6 mM MgCl₂, 15 units of *Bam*HI enzyme and 15 units of *Scal* enzyme]. Thereafter, a *Bam*HI-*Scal* fragment of about 700 bp was obtained by subjecting the resulting reaction solution to 0.8% agarose gel electrophoresis, and the fragment was purified by glass powder method (Gene Clean™, Bio-101).

One µg of Trp-TrpE DNA which is an expression vector was digested by incubating it at 37°C for 1 hour in 20 µl of a reaction solution [150 mM NaCl, 6 mM Tris-HCl (pH 7.5), 6 mM MgCl₂, 15 units of *Bam*HI enzyme and 15 units of *Scal* enzyme]. After adding 39 µl of water, the resulting reaction solution was heat-treated at 70°C for 5 minutes, mixed with 1 µl (250 U/µl) of a bacterial alkaline phosphatase (BAP) and then incubated at 37°C for 1 hour. The reaction solution was subsequently extracted with phenol, the aqueous layer was subjected to ethanol precipitation followed by drying of the precipitate. One µg of the *Bam*HI-*Scal*-treated vector DNA obtained and the above C11-7 DNA fragment was added to 5 µl of 10 x ligase buffer [660 mM Tris-HCl (pH 7.5), 66 mM MgCl₂, 100 mM dithiothreitol and 1 mM ATP] and 1 µl of T4 DNA ligase (350 U/µl), and water was then added to the mixture to 50 µl of the final volume. Thereafter, the thus prepared mixture was incubated overnight at 16°C to complete ligation.

E. coli HB101 strain was transformed with 10 µl of the resulting reaction solution. Competent *E. coli* strain for use in the transformation was prepared by calcium chloride technique [Mandel, M. and Higa, A.; *J. Mol. Biol.*, 53, 159 - 162 (1970)]. The transformed *E. coli* strain cells were spread on an LB-plate (1% trypton, 0.5% yeast extracts, 0.5% NaCl and 1.5% agar) containing 25 µg/ml of ampicillin and incubated overnight at 37°C. One loopful of each colony grown on the plate was transferred into a liquid LB medium containing 25 µg/ml of ampicillin and cultured overnight at 37°C. Cells in 1.5 ml of the cultured medium were collected by centrifugation, and Miniprep of plasmid DNA was carried out by alkali method (Maniatis *et al*; *Molecular Cloning: A Laboratory Manual*, 1982). One µg of the plasmid DNA obtained was digested at 37°C for 1 hour in 20 µl of a reaction solution [150 mM NaCl, 6 mM Tris-HCl (pH 7.5), 6 mM MgCl₂, 15 units of *Bam*HI and 15 units of *Scal*]. Thereafter, the digested solution was subjected to agarose gel electrophoresis to obtain an expression plasmid Trp-TrpE-C11-7 which can produce the 700 bp *Bam*HI-*Scal* fragment. This plasmid was transformed into *E. coli* HB101 strain and deposited on July 6, 1990 with Fermentation Research Institute, 1-3 Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305, Japan, under the Accession Number FERM P-11590 (named *E. coli* HB101/Trp-TrpE-C11-7). This deposition was subsequently converted on June 13, 1991 to an international deposition under Budapest Treaty by the same depositary institution as an international depositary authority set forth in Budapest Treaty to be given the new Accession Number FERM BP-3443.

(ii) Expression and purification of polypeptide encoded by clone C11-7:

E. coli HB101 strain transformed with the expression plasmid Trp-TrpE-C11-7 was inoculated into 3 ml of a liquid 2YT medium (1.6% trypton, 1% yeast extracts and 0.5% NaCl) containing 50 µg/ml of ampicillin and cultured at 37°C for 9 hours. One ml portion of the cultured broth was inoculated into 100 ml of a liquid M9-CA medium (0.6% Na₂HPO₄, 0.5% KH₂PO₄, 0.5% NaCl, 0.1% NH₄Cl, 0.1 mM CaCl₂, 2 mM MgSO₄, 0.5% casamino acid and 0.2% glucose) containing 50 µg/ml of ampicillin and cultured at 37°C for 21 hours. A 18-ml portion of the resulting culture broth was then inoculated into 1.2 l of the M9-CA medium and cultured at 37°C. When turbidity at OD₆₀₀ of the culture broth reached 0.3, indole acrylate was added to a final concentration of 40 mg/l, and the culturing was continued for additional 16 hours. Cells collected from the final culture broth by centrifugation were suspended in 20 ml of buffer A [50 mM Tris-HCl (pH 8.0), 1 mM EDTA and 30 mM NaCl] and the cell suspension was again subjected to centrifugation to obtain 2.6 g of expressed cells. The thus obtained cells were suspended in 10 ml of the buffer A, disintegrated by ultrasonic treatment, and then subjected to centrifugation to obtain an insoluble fraction containing a fused polypeptide of TrpE with a polypeptide which is encoded by the non-A non-B type hepatitis virus cDNA. The fused polypeptide in the insoluble fraction was solubilized and extracted using 10 ml of the buffer A containing 9 M urea. Thereafter, the solubilized extract was subjected to an S-Sepharose ion exchange column chromatography with an NaCl gradient of from 0 M to 0.5 M to purify the fused polypeptide.

(iii) Construction of expression plasmid Trp-TrpE-C11-C21:

The clone C11-C21 was expressed as a fused polypeptide with TrpE in *E. coli* under the control of a promoter (see Fig. 20).

Firstly, 1 ng of plasmid pUC-C11-C21 DNA which has been obtained by incorporating C11-C21 clone into pUC119 was subjected to PCR method using two primers (5'-TTACGAATTCATGGGCACGAATCCT-3' and 5'-TTAATCGATGACCTTACCCACATTGCG-3'). PCR method was carried out using Gene Amp™ kit (DNA Amplification Reagent Kit, Perkin Elmer Cetus) under reaction conditions of: DNA denaturation, 95°C for 1.5 minutes; annealing, 50°C for 2 minutes; and DNA synthesis, 70°C for 3 minutes. DNA fragments thus obtained were

separated by 0.8% agarose gel electrophoresis and purified by glass powder technique. Separately from this, pUC118 was digested with a restriction enzyme *Sma*I and then ligated with the DNA fragment obtained by PCR method in a buffer solution containing T4 ligase to obtain a plasmid pUC118-C11-C21-*Sma*. One μ g of the plasmid DNA obtained was digested at 37°C for 1 hour in a restriction enzyme reaction solution [150 mM NaCl, 6 mM Tris-HCl (pH 7.9), 6 mM MgCl₂, 15 units of *Eco*RI enzyme and 15 units of *Bam*HI enzyme]. Thereafter, the resulting reaction mixture was subjected to 0.8% agarose gel electrophoresis to isolate an *Eco*RI-*Bam*HI fragment of about 380 bp which was then purified by glass powder technique (Gene Clean™, Bio-101).

Next, ligation and transformation were carried out substantially in the same manner as in the aforementioned procedure (i) except that restriction digestion of the expression vector Trp-TrpE DNA was carried out using *Eco*RI and *Bam*HI instead of *Bam*HI and *Sca*I. Thereafter, an expression plasmid Trp-TrpE-C11-C21 which can produce the *Eco*RI-*Bam*HI fragment of about 380 bp was selected by agarose gel electrophoresis purification. This plasmid was transformed into *E. coli* HB101 strain and deposited on December 11, 1990 with Fermentation Research Institute, Agency of Industrial Science and Technology, the same address, under the Accession Number FERM P-11893 (named *E. coli* HB101/Trp-TrpE-C11-C21). The deposition was also subsequently converted on June 17, 1991 to an international deposition under Budapest Treaty by the same depositary institution as an international depositary authority set forth in Budapest Treaty to be given the Accession Number FERM BP-3451.

(iv) Expression and purification of polypeptide encoded by clone C11-C21:

Expression and purification of a fused polypeptide were carried out substantially in the same manner as in the aforementioned procedure (ii), except that the expression plasmid Trp-TrpE-C11-C21 obtained by the above procedure (iii) was used instead of Trp-TrpE-C11-7.

Example 6

Measurement of anti-non-A non-B type hepatitis virus antibody in serum from non-A non-B hepatitis patient

(i) Measurement by western blotting:

The expressed product obtained and purified in Example 5 was subjected in turn to SDS-polyacrylamide gel electrophoresis [Laemmli; *Nature*, 277, 680 (1970)] and to blotting on a nitrocellulose filter (Bio-Rad, Trans-blot) in usual way. The filter was blocked with a 3% gelatin solution and then reacted with each serum samples from normal persons or non-A non-B hepatitis patients. After washing, the resulting filter was reacted with a peroxidase-labeled human IgG (goat antibody). Thereafter, the filter was washed again and soaked in a solution containing diaminobenzidine as reaction substrate to confirm color development.

The results are shown in Figs. 21 and 22. In Fig. 21, the expressed polypeptide TrpE-C11-7 (group 1) obtained in Example 5-(ii) was used as antigen, and in Fig. 22, the expressed polypeptide TrpE-C11-C21 (group 2) in Example 5-(iv) was used. In each case, no reaction was observed with a normal serum sample, but a strong reaction with a patient's serum sample was found with a specific band.

(ii) Measurement by enzyme-linked immunosorbent assay (ELISA) :

ELISA can be used as a means to make diagnosis of a large number of serum samples as compared to the case of western blotting method. ELISA was carried out as follows:

A purified antigen sample was diluted with PBS(-) to a concentration of 5 μ g/ml and fixed to a micro-plate at 4°C or room temperature. After washing several times with a washing solution, a diluted serum sample to be detected was added to the resulting plate and incubated for 1 hour at 37°C or room temperature. After washing, peroxidase-labeled anti-human IgG (goat antibody) was added and incubated at 37°C or room temperature to complete the reaction. After washing several times, 50 μ l of a diaminobenzidine solution was added and incubated at 37°C to develop color. Thereafter, the coloring reaction was stopped with 2 M H₂SO₄ and the color was measured by a colorimeter.

Positive ratios in the case of the use of the expressed polypeptide antigens, TrpE-C11-7 (group 1) and TrpE-C11-C21 (group 2), of the present invention were compared with the case of the use of a commercially available kit of Chiron Corp. (Ortho HCV Ab ELISA Test). As shown in Table 1, the use of the Chiron's kit resulted in 69.7% of the positive ratio, while positive ratios in the case of the use of the TrpE-C11-7 and TrpE-C11-C21 were 78.8% and 84.8%, respectively. Moreover, the positive ratio increased to 98.9% (30 of 31 cases) when these two expressed polypeptides of the present invention were used in combination (see Fig. 23).

Example 7

Detection of non-A non-B type hepatitis virus group 2 gene in blood plasma from non-A non-B hepatitis patient by RT-PCR

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RT-PCR was carried out as follows:

To 100 μ l of a blood plasma sample collected from a non-A non-B hepatitis patient was added 300 μ l of a 6 M GTC solution (6 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% sarcosyl and 0.2 M 2-mercaptoethanol), and the mixture was stirred. To this were further added 40 μ l of 2 M sodium acetate (pH 5.2), 400 μ l of phenol and 80 μ l of chloroform/isoamyl alcohol (49:1), and then thoroughly stirred. Aqueous solution layer separated from the mixture was mixed with isopropyl alcohol and then subjected to centrifugation. Synthesis of cDNA was carried out using the pellet as a source of RNA. For the cDNA synthesis, an RNase inhibitor and a reverse transcriptase were added to a reaction solution containing 10 mM Tris-HCl, 0.01% gelatin, 1 mM each dNTP, 4 mM $MgCl_2$, 1 mM DTT and 100 pmole each primer, and the mixture was incubated at 37°C for 2 hours to complete the reaction. Then, PCR was carried out using the cDNA obtained. In order to increase sensitivity and specificity for the detection of bands, a two step PCR method was employed, that is, first PCR using two primers (1st step PCR) and subsequent PCR using two primers which exist inside the first PCR product (2nd step PCR). For the PCR reaction, each amplification cycle was carried out using 100 μ l of a reaction solution containing cDNA, 10 mM Tris-HCl, 0.01% gelatin, 2 mM each dNTP, 1.5 mM $MgCl_2$ and 50 pmol each primer, under reaction conditions of: denaturation, 94°C for 1.5 minutes; annealing, 50°C for 2 minutes; and chain elongation, 70°C for 2 minutes. The amplification cycle was repeated 35 times. Effects of several primers were evaluated. As the results, it was found that the group 2-specific DNA fragments are capable of being detected by the use of the following 4 primers:

1st step PCR

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kk21: 5'-GGATACACCGGTGACTTTGA-3'

kk22: 5'-TGCATGCACGTGGCGATGTA-3'

2nd step PCR

kk26: 5'-GATGCCCCACTTCCTCTCCCA-3'

kk27: 5'-GTCAGGGTAACCTCGTTGGT-3'

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By applying these 4 primers to the PCR method, a DNA fragment of 206 bp can be detected. As a control, primers were synthesized from the base sequence of J1 and detection of group 1 DNA fragments was attempted. Results of the PCR from blood plasma samples of non-A non-B hepatitis patient are shown in Table 2.

It was known that DNA fragments from the non-A non-B hepatitis virus can be detected by both the PCRs using group 1 primers (i.e., group 1 PCR) and group 2 primers (i.e., group 2 PCR), and therefore two samples, Nos. 3 and 5, which are considered to include both groups 1- and 2-relating viruses were sequenced for their viral genes. As shown in Table 3, when nucleotide sequences of DNA fragments obtained by group 2 PCR were compared with C10-13 which is a group 2 clone, homologies of 85% and 88% were observed, indicating effective detection of group 2 genes. When these two nucleotide sequences were compared with the aforementioned group 1 clone J1 (Miyamura et al, supra), only 64.8% and 68% homologies were observed. Results of the homology evaluation indicate that the primers used in the group 2 PCR can selectively detect group 2 viral genes.

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Table 1

Sample No.	TrpE·C11-7 (group 1)	TrpE·C11-C21 (group 2)	Kit of Chiron Corp. (group 1)
1	+++	+++	+++
2	+++	+++	++
3	+	+++	+++
4	+++	+	+++
5	-	+++	+++
6	+++	+	++
7	+++	+++	+++
8	+++	+++	+++
9	+++	++	+++
10	±	±	-
11	+++	-	++
12	+++	+++	-
13	+++	-	+
14	+++	+++	+++
15	+++	+++	+++
16	+++	+	+++
17	-	-	-
18	+	+	-
19	+	+++	-
20	++	++	+++
21	+++	+++	++
22	-	++	-
23	-	-	-
24	+	+	+
25	+++	+++	+++
26	-	++	-
27	+++	+++	++
28	+	+	+++
29	+++	+++	+++
30	+++	++	+++
31	-	++	+++
32	-	++	-
33	+	-	-
NK	-	-	-
NP	-	-	-

Note: NK and NP are negative controls.

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Table 2

Sample No.	Group 1 PCR	Group 2 PCR
1	+	-
2	+	±
10 3	+	+
4	+	-
5	+	+
15 6	+	-
7	+	-
8	+	-
9	+	-
20 10	+	-
11	+	+
13	-	+
42	-	+
25 169	+	+
260	-	+
244	-	-
30 248	-	+
NC	-	-

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Table 3

Sample No.	Nucleotide homology with clone C10-13
3	85%
5	88%

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As seen from the foregoing examples, the present invention has the following advantages:

The cDNA sequences according to the present invention are specific to non-A non-B hepatitis, and polypeptides which are produced by incorporating these genes into a protein expression system in microbial host cells such as *E. coli* can react immunologically with sera samples from a number of non-A non-B hepatitis patients, whereby a kit for diagnosing non-A non-B hepatitis is capable of preparing with markedly high sensitivity and judging accuracy. Also, it is possible to make diagnosis of this disease using said sequences as a probe directly or other probes with higher specificity synthesized on the basis of the sequences. In addition, not only diagnosis of the disease but also isolation of non-A non-B hepatitis-specific genes can be accomplished by employing a gene amplification method (PCR method).

Sequence Listing

5

SEQ ID NO:1

SEQUENCE LENGTH:763 base pairs

10

SEQUENCE TYPE:nucleic acid

STRANDEDNESS:double

TOPOLOGY:linear

MOLECULE TYPE:cDNA to genomic RNA

15

CG CAG TCA TTC CAA GTG GCC CAT CTA CAC GCT CCC ACT GGC AGC GGC 47
 Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly Ser Gly
 1 5 10 15

20

AAG AGT ACT AAA GTG CCG GCT GCA TAT GCC AGC CAA GGG TAC AAG GTG 95
 Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ser Gln Gly Tyr Lys Val
 20 25 30

25

CTC GTC CTC AAC CCG TCC GTT GCC GCC ACC TTA GGT TTT GGA GCG TAT 143
 Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr
 35 40 45

30

ATG TCT AAG GCA CAT GGC ACC GAC CCC AAC ATC AGA ACT GGG GTA AGG 191
 Met Ser Lys Ala His Gly Thr Asp Pro Asn Ile Arg Thr Gly Val Arg
 50 55 60

35

ACT ATC ACC ACA GGC GCC CCC ATC ACG TAC TCC ACC TAC GGC AAG TTC 239
 Thr Ile Thr Thr Gly Ala Pro Ile Thr Tyr Ser Thr Tyr Gly Lys Phe
 65 70 75

40

CTT GCC GAC GGT GGT TGT TCT GGG GGC GCT TAT GAC ATC ATA ATG TGT 287
 Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Met Cys
 80 85 90 95

45

GAT GAG TGC CAC TCA ACT GAC GCG ACT TCC ATC TTG GGC ATC GGC ACG 335
 Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile Gly Thr
 100 105 110

50

GTC CTG GAC CAA GCG GAG ACG GCT GGA GCA CGG CTC GTC GTG CTC GCC 383
 Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala
 115 120 125

55

5 ACC GCT ACG CCT CCG GGA TCG GTC ACC GTG CCA CAC CCG AAT ATT GAG 431
 Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu
 130 135 140

10 GAG GTG GCC CTG TCT AAC ACT GGA GAG ATC CCC TTC TAT GGC AAA GGC 479
 Glu Val Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr Gly Lys Gly
 145 150 155

15 ATC CCC ATT GAA GTC ATC AAG GGG GGA AGG CAT CTC ATT TTC TGC CAT 527
 Ile Pro Ile Glu Val Ile Lys Gly Gly Arg His Leu Ile Phe Cys His
 160 165 170 175

20 TCC AAG AAG AAG TGC GAC GAG CTC GCC GCG AAG TTG TCA GGC CTC GGC 575
 Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly Leu Gly
 180 185 190

25 ATT AAT GCT GTG GCA TAC TAC CGG GGT CTT GAT GTG TCC GTC ATA CCG 623
 Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro
 195 200 205

30 ACC AGC GGA GAC GTC GTT GTC GTG GCA ACA GAC GCT CTA ATG ACG GGC 671
 Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met Thr Gly
 210 215 220

35 TAT ACC GGC GAT TTT GAC TCA GTG ATC GAC TGT AAC ACA TGC GTC ACC 719
 Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr
 225 230 235

40 CAG ACA GTC GAC TTC AGC TTG GAC CCC ACC TTC ACC ATT GAG AC 763
 Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
 240 245 250

45 SEQ ID NO:2
 SEQUENCE LENGTH:615 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double

50 TOPOLOGY:linear
 MOLECULE TYPE:cdna to genomic RNA

55 C ACG CCC GGT TTG CCC GTG TGT CAA GAC CAC CTG GAG TTC TGG GAA GCG 49
 Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Ala
 1 5 10 15

5	GTC TTC ACA GGT CTC ACG CAC ATT GAT GCC CAC TTC CTC TCC CAG ACA Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu Ser Gln Thr 20 25 30	97
10	AAG CAA GGA GGA GAC AAC TTC GCG TAT CTA ACG GCC TAC CAG GCC ACA Lys Gln Gly Gly Asp Asn Phe Ala Tyr Leu Thr Ala Tyr Gln Ala Thr 35 40 45	145
15	GTG TGC GCT AGG GCA AAG GCC CCT CCT CCC TCG TGG GAT GTG ATG TGG Val Cys Ala Arg Ala Lys Ala Pro Pro Pro Ser Trp Asp Val Met Trp 50 55 60	193
20	AAA TGT CTA GCT AGG CTG AAG CCT ACA CTA ATT GGT CCT ACC CCC CTC Lys Cys Leu Ala Arg Leu Lys Pro Thr Leu Ile Gly Pro Thr Pro Leu 65 70 75 80	241
25	CTG TAC CGC TTG GGT GCC GTG ACC AAC GAG GTT ACC CTG ACG CAC CCC Leu Tyr Arg Leu Gly Ala Val Thr Asn Glu Val Thr Leu Thr His Pro 85 90 95	289
30	GTG ACG AAA TAC ATC GCC ACG TGC ATG CAA GCT GAC CTC GAG ATC ATG Val Thr Lys Tyr Ile Ala Thr Cys Met Gln Ala Asp Leu Glu Ile Met 100 105 110	337
35	ACG AGC ACA TGG GTC CTA GCA GGG GGG GTG CTA GCC GCC GTG GCA GCT Thr Ser Thr Trp Val Leu Ala Gly Gly Val Leu Ala Ala Val Ala Ala 115 120 125	385
40	TAC TGC CTG GCA ACC GGC TGT GTT TCC ATC ATC GGC CGC CTA CAC CTG Tyr Cys Leu Ala Thr Gly Cys Val Ser Ile Ile Gly Arg Leu His Leu 130 135 140	433
45	AAT GAT CAA GTG GTT GTG ACT CCT GAC AAA GAA ATC TTA TAT GAG GCC Asn Asp Gln Val Val Val Thr Pro Asp Lys Glu Ile Leu Tyr Glu Ala 145 150 155 160	481
50	TTT GAT GAG ATG GAA GAA TGC GCC TCC AAA GCC GCC CTC ATT GAG GAA Phe Asp Glu Met Glu Gln Cys Ala Ser Lys Ala Ala Leu Ile Glu Glu 165 170 175	529
55	GGG CAG CGG ATG GCG GAG ATG CTC AAG TCT AAG ATA CAA GGC CTC CTA Gly Gln Arg Met Ala Glu Met Leu Lys Ser Lys Ile Gln Gly Leu Leu 180 185 190	577

CAA CAG GCC ACA AGA CAG GCC CAA GAC ATA CAG CCA GC 615
 Gln Gln Ala Thr Arg Gln Ala Gln Asp Ile Gln Pro
 5 195 200

SEQ ID NO:3
 10 SEQUENCE LENGTH:771 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 TOPOLOGY:linear
 15 MOLECULE TYPE:cDNA to genomic RNA

GT GAG CGA GCC TCA GGA ATG TTT GAC AGT GTA GTG CTC TGT GAG TGC 47
 Glu Arg Ala Ser Gly Met Phe Asp Ser Val Val Leu Cys Glu Cys
 20 1 5 10 15

TAT GAC GCA GGG GCT GCA TGG TAC GAG CTT ACA CCA GCG GAG ACC ACC 95
 Tyr Asp Ala Gly Ala Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr
 25 20 25 30

GTC AGG CTC AGA GCG TAT TTC AAC ACA CCT GGC TTG CCT GTG TGT CAA 143
 Val Arg Leu Arg Ala Tyr Phe Asn Thr Pro Gly Leu Pro Val Cys Gln
 30 35 40 45

GAC CAT CTT GAG TTC TGG GAG GCA GTT TTC ACC GGC CTC ACA CAC ATA 191
 Asp His Leu Glu Phe Trp Glu Ala Val Phe Thr Gly Leu Thr His Ile
 35 50 55 60

GAT GCC CAC TTC CTT TCC CAG ACA AAG CAA GCA GGG GAC AAT TTC GCA 239
 Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Ala
 40 65 70 75

TAC TTG ACA GCC TAC CAG GCT ACA GTG TGC GCC AGA GCC AAA GCC CCT 287
 Tyr Leu Thr Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Lys Ala Pro
 45 80 85 90 95

CCC CCG TCC TGG GAC GTC ATG TGG AAG TGC CTG ACT CGG CTC AAG CCC 335
 Pro Pro Ser Trp Asp Val Met Trp Lys Cys Leu Thr Arg Leu Lys Pro -
 50 100 105 110

ACG CTT GTG GCC CCT ACA CCC CTT CTG TAC CGT TTA GGC TCT GTT ACT 383
 Thr Leu Val Ala Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ser Val Thr
 55 115 120 125

5	AAC GAG GTC ACC CTC ACA CAT CCT GTG ACG AAA TAC ATC GCC ACT TGC Asn Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Ala Thr Cys 130 135 140	431
10	ATG CAA GCT GAC CTT GAG GTC ATG ACC AGC ACG TGG GTC CTA GCT GGG Met Gln Ala Asp Leu Glu Val Met Thr Ser Thr Trp Val Leu Ala Gly 145 150 155	479
15	GGG GTC TTG GCA GCC GTC GCC GCG TAT TGC CTG GCG ACT GGG TGT GTC Gly Val Leu Ala Ala Val Ala Ala Tyr Cys Leu Ala Thr Gly Cys Val 160 165 170 175	527
20	TCC ATC ATC GGC CGC TTG CAC ATC AAT CAG CGA GCC GTC GTT GCA CCA Ser Ile Ile Gly Arg Leu His Ile Asn Gln Arg Ala Val Val Ala Pro 180 185 190	575
25	GAC AAG GAG GTC CTT TAT GAG GCT TTT GAT GAG ATG GAG GAG TGT GCC Asp Lys Glu Val Leu Tyr Glu Ala Phe Asp Glu Met Glu Glu Cys Ala 195 200 205	623
30	TCT AAA GCG GCT CTC ATT GAA GAG GGG CAG CGG ATA GCC GAG ATG CTG Ser Lys Ala Ala Leu Ile Glu Glu Gly Gln Arg Ile Ala Glu Met Leu 210 215 220	671
35	AAG TCC AAG ATC CAA GGC TTA TTG CAG CAA GCC TCT AAA CAG GCC CAG Lys Ser Lys Ile Gln Gly Leu Leu Gln Gln Ala Ser Lys Gln Ala Gln 225 230 235	719
40	GAC ATA CAA CCC GCT GTG CAG CCT CAT GGC CCA AGG TGG AGC AAT TCT Asp Ile Gln Pro Ala Val Gln Pro His Gly Pro Arg Trp Ser Asn Ser 240 245 250 255	767
45	GGG C Gly	771

50 SEQ ID NO:4
 SEQUENCE LENGTH:630 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 TOPOLOGY:linear
 55 MOLECULE TYPE:cDNA to genomic RNA

5	C TGG TAT GAA CTT ACG CCT GCT GAG ACT ACG GTG AGA CTC CGG GCC TAT Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val Arg Leu Arg Ala Tyr	49
	1 5 10 15	
10	TTC AAC ACG CCC GGC CTG CCT GTG TGT CAA GAC CAC CTG GAA TTC TGG Phe Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu Phe Trp	97
	20 25 30	
15	GAG GCG GTC TTC ACA GGT CTC ACA CAC ATC GAT GCC CAC TTC CTC TCC Glu Ala Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu Ser	145
	35 40 45	
20	CAG ACG AAG CAA GGA GGA GAT AAC TTT GCA TAT TTA ACA GCC TAC CAG Gln Thr Lys Gln Gly Gly Asp Asn Phe Ala Tyr Leu Thr Ala Tyr Gln	193
	50 55 60	
25	GCC ACA GTC TGC GCT AGG GCA AAG GCT CCC CCT CCT TCG TGG GAC GTG Ala Thr Val Cys Ala Arg Ala Lys Ala Pro Pro Pro Ser Trp Asp Val	241
	65 70 75 80	
30	ATG TGG AAG TGT TTG ATT AGG CTC AAA CCT ACA CTG ACT GGT CCT ACC Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu Thr Gly Pro Thr	289
	85 90 95	
35	CCC CTC CTG TAC CGC TTG GGT GCC GTG ACC AAC GAG GTT ACC CTG ACT Pro Leu Leu Tyr Arg Leu Gly Ala Val Thr Asn Glu Val Thr Leu Thr	337
	100 105 110	
40	CAC CCC ATG ACG AAA TAT ATC GCC ACT TGT ATG CAA GCT GAT CTT GAG His Pro Met Thr Lys Tyr Ile Ala Thr Cys Met Gln Ala Asp Leu Glu	385
	115 120 125	
45	ATC ATG ACA AGC ACA TGG GTC TTG GCG GGG GGG GTG CTA GCC GCT GTG Ile Met Thr Ser Thr Trp Val Leu Ala Gly Gly Val Leu Ala Ala Val	433
	130 135 140	
50	GCA GCT TAC TGC CTA GCG ACC GGC TGC ATT TCC ATC ATT GGC CGC CTT Ala Ala Tyr Cys Leu Ala Thr Gly Cys Ile Ser Ile Ile Gly Arg Leu	481
	145 150 155 160	
55	CAC CTG AAT GAT CGG GTG GTC GTG ACC CCT GAT AAG GAA ATT TTA TAT His Leu Asn Asp Arg Val Val Val Thr Pro Asp Lys Glu Ile Leu Tyr	529
	165 170 175	

5 GAG GCC TTT GAT GAG ATG GAA GAG TGC GCC TCC AAA GCC GCC CTC ATT 577
 Glu Ala Phe Asp Glu Met Glu Glu Cys Ala Ser Lys Ala Ala Leu Ile
 180 185 190

10 GAG GAA GGG CAG CGG ATG GCG GAG ATG CTG AAG TCT AAA ATA CAA GGC 625
 Glu Glu Gly Gln Arg Met Ala Glu Met Leu Lys Ser Lys Ile Gln Gly
 195 200 205

15 CTC TT 630
 Leu

20 SEQ ID NO:5
 SEQUENCE LENGTH:1426 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 TOPOLOGY:linear
 MOLECULE TYPE:cDNA to genomic RNA

25 GGG ATC AAC CCT AAC ATC AGG ACC GGA GTA CGG ACC GTG ACC ACC GGG 48
 Gly Ile Asn Pro Asn Ile Arg Thr Gly Val Arg Thr Val Thr Thr Gly
 1 5 10 15

30 GAC TCC ATC ACC TAC TCC ACT TAT GGC AAG TTT ATC GCA GAT GGA GGT 96
 Asp Ser Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Ile Ala Asp Gly Gly
 20 25 30

35 TGC GCA CAT GGT GCC TAT GAC GTC ATC ATA TGC GAC GAA TGC CAT TCA 144
 Cys Ala His Gly Ala Tyr Asp Val Ile Ile Cys Asp Glu Cys His Ser
 35 40 45

40 GTG GAC GCT ACT ACC ATC CTT GGC ATT GGA ACA GTC CTT GAC CAG GCT 192
 Val Asp Ala Thr Thr Ile Leu Gly Ile Gly Thr Val Leu Asp Gln Ala
 50 55 60

45 GAG ACC GCA GGT GCC AGG CTA GTG GTT TTA GCC ACA GCC ACG CCA CCC 240
 Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr Ala Thr Pro Pro
 65 70 75 80

50 GGT ACG GTA ACA ACT CCC CAC GCT AAC ATA GAG GAG GTG GCC CTT GGT 288
 Gly Thr Val Thr Thr Pro His Ala Asn Ile Glu Glu Val Ala Leu Gly
 85 90 95

5	CAC GAA GGC GAG ATT CCT TTT TAT GGC AAG GCT ATT CCC CTA GCT TTC His Glu Gly Glu Ile Pro Phe Tyr Gly Lys Ala Ile Pro Leu Ala Phe 100 105 110	336
10	ATC AAG GGG GGC AGA CAC CTA ATT TTT TGC CAT TCA AAG AAG AAG TGC Ile Lys Gly Gly Arg His Leu Ile Phe Cys His Ser Lys Lys Lys Cys 115 120 125	384
15	GAC GAG CTC GCA GCA GCC CTT CGG GGC ATG GGT ATC AAT GCC GTT GCC Asp Glu Leu Ala Ala Ala Leu Arg Gly Met Gly Ile Asn Ala Val Ala 130 135 140	432
20	TAC TAC AGG GGT CTC GAC GTC TCC GTT ATA CCA ACT CAA GGA GAC GTG Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro Thr Gln Gly Asp Val 145 150 155 160	480
25	GTG GTT GTC GCC ACC GAT GCC CTA ATG ACT GGA TAC ACC GGT GAC TTT Val Val Val Ala Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe 165 170 175	528
30	GAC TCT GTC ATC GAC TGC AAC GTT GCA GTC ACT CAG ATT GTT GAC TTT Asp Ser Val Ile Asp Cys Asn Val Ala Val Thr Gln Ile Val Asp Phe 180 185 190	576
35	AGC CTA GAC CCA ACT TTT ACC ATC ACC ACT CAA ACC GTC CCT CAG GAG Ser Leu Asp Pro Thr Phe Thr Ile Thr Thr Gln Thr Val Pro Gln Glu 195 200 205	624
40	GCT GTC TCC CGT AGT CAA CGT AGA GGG AGA ACT GGG AGG GGG CGA CTG Ala Val Ser Arg Ser Gln Arg Arg Gly Arg Thr Gly Arg Gly Arg Leu 210 215 220	672
45	GGC ACT TAC AGG TAT GTC TCG TCA GGC GAG AGG CCG TCT GGG ATG TTC Gly Thr Tyr Arg Tyr Val Ser Ser Gly Glu Arg Pro Ser Gly Met Phe 225 230 235 240	720
50	GAC AGC GTA GTA CTC TGC GAG TGC TAT GAT GCC GGG GCA GCC TGG TAC Asp Ser Val Val Leu Cys Glu Cys Tyr Asp Ala Gly Ala Ala Trp Tyr 245 250 255	768
55	GAG CTT ACA CCT GCT GAG ACC ACA GTG AGA CTC CGG GCT TAT TTC AAC Glu Leu Thr Pro Ala Glu Thr Thr Val Arg Leu Arg Ala Tyr Phe Asn 260 265 270	816

5	ACG CCC GGT TTG CCC GTG TGT CAA GAC CAC CTG GAG TTC TGG GAA GCG Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Ala 275 280 285	864
10	GTC TTC ACA GGT CTC ACG CAC ATT GAT GCC CAC TTC CTC TCC CAG ACA Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu Ser Gln Thr 290 295 300	912
15	AAG CAA GGA GGA GAC AAC TTC GCG TAT CTA ACG GCC TAC CAG GCC ACA Lys Gln Gly Gly Asp Asn Phe Ala Tyr Leu Thr Ala Tyr Gln Ala Thr 305 310 315 320	960
20	GTG TGC GCT AGG GCA AAG GCC CCT CCT CCC TCG TGG GAT GTG ATG TGG Val Cys Ala Arg Ala Lys Ala Pro Pro Pro Ser Trp Asp Val Met Trp 325 330 335	1008
25	AAA TGT CTA GCT AGG CTG AAG CCT ACA CTA ATT GGT CCT ACC CCC CTC Lys Cys Leu Ala Arg Leu Lys Pro Thr Leu Ile Gly Pro Thr Pro Leu 340 345 350	1056
30	CTG TAC CGC TTG GGT GCC GTG ACC AAC GAG GTT ACC CTG ACG CAC CCC Leu Tyr Arg Leu Gly Ala Val Thr Asn Glu Val Thr Leu Thr His Pro 355 360 365	1104
35	GTG ACG AAA TAC ATC GCC ACG TGC ATG CAA GTG AAC CTC GAG ATC ATG Val Thr Lys Tyr Ile Ala Thr Cys Met Gln Val Asn Leu Glu Ile Met 370 375 380	1152
40	ACG AGC ACA TGG GTC CTA GCA GGG GGG GTG CTA GCC GCC GTG GCA GCT Thr Ser Thr Trp Val Leu Ala Gly Gly Val Leu Ala Ala Val Ala Ala 385 390 395 400	1200
45	TAC TGC CTG GCA ACC GGC TGT GTT TCC ATC ATC GGC CGC CTA CAC CTG Tyr Cys Leu Ala Thr Gly Cys Val Ser Ile Ile Gly Arg Leu His Leu 405 410 415	1248
50	AAT GAT CAA GTG GTT GTG ACT CCT GAC AAA GAA ATC TTA TAT GAG GCC Asn Asp Gln Val Val Val Thr Pro Asp Lys Glu Ile Leu Tyr Glu Ala 420 425 430	1296
55	TTT GAT GAG ATG GAA GAA TGC GCC TCC AAA GCC GCC CTC ATT GAG GAA Phe Asp Glu Met Glu Glu Cys Ala Ser Lys Ala Ala Leu Ile Glu Glu 435 440 445	1344

5 GGG CAG CGG ATG GCG GAG ATG CTC AAG TCT AAG ATA CAA GGC CTC CTA 1392
 Gly Gln Arg Met Ala Gln Met Leu Lys Ser Lys Ile Gln Gly Leu Leu
 450 455 460

10 CAA CAG GCC ACA AGA CAG GCC CAA GAC ATA CAG C 1426
 Gln Gln Ala Thr Arg Gln Ala Gln Asp Ile Gln
 465 470 475

15 SEQ ID NO:6
 SEQUENCE LENGTH:855 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 20 TOPOLOGY:linear
 MOLECULE TYPE:cDNA to genomic RNA

25 CG CAG ACA TTC CAA GTG GCC CAT CTG CAC GCT CCC ACT GGT AGC GGC 47
 Gln Thr Phe Gln Val Ala His Leu His Ala Pro Thr Gly Ser Gly
 1 5 10 15

30 AAG AGC ACT AAG GTG CCG GCT GCA TAT GCG GCC CAA GGG TAC AAG GTA 95
 Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val
 20 25 30

35 CTC GTC CTG AAC CCG TCC GTT GCC GCC ACT TTA GCC TTT GGG GCG TAC 143
 Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Ala Phe Gly Ala Tyr
 35 40 45

40 ATG TCT AAG GCA CAT GGT GTC GAC CCT AAC ATC AGA ACT GGG GTG AGG 191
 Met Ser Lys Ala His Gly Val Asp Pro Asn Ile Arg Thr Gly Val Arg
 50 55 60

45 ACC ATC ACC ACG GGC GCT CCC ATC ACG TAC TCC ACC TAT GGT AAG TTC 239
 Thr Ile Thr Thr Gly Ala Pro Ile Thr Tyr Ser Thr Tyr Gly Lys Phe
 65 70 75

50 CTT GCC GAC GGT GGT TGC TCT GGG GGC GCC TAT GAC ATC ATA ATA TGT 287
 Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys
 80 85 90 95

55 GAT GAG TGC CAC TCA ACT GAC TCG ACA TCC ATC TTG GGC ATC GGC ACA 335
 Asp Glu Cys His Ser Thr Asp Ser Thr Ser Ile Leu Gly Ile Gly Thr
 100 105 110

5	GTC CTG GAC CAA GCG GAG ACG GCT GGA GCG CGG CTC GTC GTG CTC GCT Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala 115 120 125	383
10	ACC GCT ACG CCT CCG GGA TCG GTC ACC GTG CCA CAT CCC AAT ATC GAG Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu 130 135 140	431
15	GAG GTG GCC CTG TCC ACC ACT GGA GAG ATT CCC TTC TAC GGC AAA GCT Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala 145 150 155	479
20	ATC CCC ATC GAG ACA ATC AAG GGG GGG AGG CAT CTC ATC TTC TGC CGT Ile Pro Ile Glu Thr Ile Lys Gly Gly Arg His Leu Ile Phe Cys Arg 160 165 170 175	527
25	TCC AAG AAG AAG TGT GAC GAG CTC GCT GGA AAG CTG TCA GCC CTC GGA Ser Lys Lys Lys Cys Asp Glu Leu Ala Gly Lys Leu Ser Ala Leu Gly 180 185 190	575
30	ATC AAC GCT GTA GCG TAC TAC CGG GGT CTT GAT GTA TCC GTC ATA CCG Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro 195 200 205	623
35	ACC AGC GGA GAC GTC GTT GTC GTG GCA ACA GAC GCT CTA ATG ACG GGC Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met Thr Gly 210 215 220	671
40	TAC ACC GGT GAC TTT GAT TCA GTG ATC GAC TGC AAT ACA TGT GTC ACC Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr 225 230 235	719
45	CAG ACA GTC GAC TTC AGC TTG GAC CCT ACC TTC ACC ATT GAG ACG ACG Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr 240 245 250 255	767
50	ACC GTG CCT CAA GAC GCG GTG TCA CGC TCG CAG CGG CGA GGC AGA ACT Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly Arg Thr - 260 265 270	815
55	GGT AGG GGT AGA GGG GGC ATA TAC AGG TTT GTG ACT CCA G Gly Arg Gly Arg Gly Gly Ile Tyr Arg Phe Val Thr Pro 275 280	855

SEQ ID NO:7

SEQUENCE LENGTH:315 base pairs

SEQUENCE TYPE:nucleic acid

STRANDEDNESS:double

TOPOLOGY:linear

MOLECULE TYPE:cDNA to genomic RNA

GAC GAG CTC GCC GCA AAG CTG TCA GGC CTC GGA GTC AAT GCT GTG GCA 48
 Asp Glu Leu Ala Ala Lys Leu Ser Gly Leu Gly Val Asn Ala Val Ala

1 5 10 15

TAC TAC CGG GGT CTC GAT GTG TCT GTC ATA CCG ACG AGC GGG GAC GTC 96
 Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro Thr Ser Gly Asp Val

20 25 30

GTT GTT GTG GCA ACA GAC GCT CTA ATG ACG GGC TAT ACC GGC GAC TTT 144
 Val Val Val Ala Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe

35 40 45

GAC TCG GTG ATC GAC TGC AAT ACA TGT GTC ACC CAA ACA GTC GAT TTC 192
 Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr Gln Thr Val Asp Phe

50 55 60

AGC TTG GAC CCT ACT TTC ACC ATT GAG ACG ACG ACC GTG CCC CAA GAC 240
 Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr Thr Val Pro Gln Asp

65 70 75 80

GCG GTG TCG CGC TCG CAG CGG CGA GGC AGG ACT GGT AGG GGC AGG GTG 288
 Ala Val Ser Arg Ser Gln Arg Arg Gly Arg Thr Gly Arg Gly Arg Val

85 90 95

GGC ATA TAC AGG TTT GTG ACT CCC GAG 315

Gly Ile Tyr Arg Phe Val Thr Pro Glu

100 105

SEQ ID NO:8

SEQUENCE LENGTH:911 base pairs

SEQUENCE TYPE:nucleic acid

STRANDEDNESS:double

TOPOLOGY:linear

MOLECULE TYPE:cDNA to genomic RNA

10 GT GAT GAG CTC GCC GCA AAG CTC TCA AGC CTC GGA CTC AAC GCT GTA 47
 Asp Glu Leu Ala Ala Lys Leu Ser Ser Leu Gly Leu Asn Ala Val
 1 5 10 15

15 GCA TAT TAC CGG GGT CTT GAT GTG TCC GTC ATA CCG ACT AGT GGA GAC 95
 Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro Thr Ser Gly Asp
 20 25 30

20 GTC GTT GTC GTG GCA ACA GAC GCT CTA ATG ACG GGC TAT ACC GGC GAC 143
 Val Val Val Val Ala Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp
 35 40 45

25 TTT GAC TCA GTG ATC GAC TGT AAC ACA TGT GTC ACC CAG ACA GTT GAT 191
 Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr Gln Thr Val Asp
 50 55 60

30 TTC AGC TTG GAT CCA ACC TTC ACC ATT GAG ACG ACG ACC GTG CCT CAA 239
 Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr Thr Val Pro Gln
 65 70 75

35 GAC GCG GTG TCG CGC TCG CAG CGG CGA GGT AGG ACT GGC AGG GGC AGG 287
 Asp Ala Val Ser Arg Ser Gln Arg Arg Gly Arg Thr Gly Arg Gly Arg
 80 85 90 95

40 GGC GGC ATC TAT AGG TTT GTG ACT CCA GGA GAA CGG CCC TCG GGC ATG 335
 Gly Gly Ile Tyr Arg Phe Val Thr Pro Gly Glu Arg Pro Ser Gly Met
 100 105 110

45 TTC GAT TCC TCG GTC CTG TGT GAG TGT TAT GAC GCG GGC TGT GCT TGG 383
 Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala Gly Cys Ala Trp
 115 120 125

50 TAT GAG CTC ACG CCC GCC GAG ACC ACG GTT AGG TTG CGG GCT TAC CTA 431
 Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val Arg Leu Arg Ala Tyr Leu
 130 135 140

55

5	AAT ACA CCA GGG TTG CCC GTC TGC CAG GAC CAT CTG GAG TTC TGG GAG Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu 145 150 155	479
10	GGC GTC TTC ACA GGC CTC ACC CAC ATA GAT GCC CAT TTC TTG TCT CAG Gly Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu Ser Gln 160 165 170 175	527
15	ACT AAG CAG GCA GGA CAC AAC TTT CCC TAC CTG GTG GCA TAC CAA GCT Thr Lys Gln Ala Gly His Asn Phe Pro Tyr Leu Val Ala Tyr Gln Ala 180 185 190	575
20	ACA GTG TGC GCC AGG GCT CAG GCT CCA CCT CCA TCG TGG GAC CAA ATG Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser Trp Asp Gln Met 195 200 205	623
25	TGG AAG TGT CTC ATA CGG CTG AAA CCT ACG CTG CAC GGG CCA ACA CCC Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly Pro Thr Pro 210 215 220	671
30	CTG CTG TAT AGG CTA GGA GCC GTG GAA AAT GAG GTC ACC CTC ACA CAC Leu Leu Tyr Arg Leu Gly Ala Val Glu Asn Glu Val Thr Leu Thr His 225 230 235	719
35	CCC ATA ACC AAA TTC ATC ATG GCA TGC ATG TCG GCT GAT CTG GAG GTC Pro Ile Thr Lys Phe Ile Met Ala Cys Met Ser Ala Asp Leu Glu Val 240 245 250 255	767
40	GTC ACC AGC ACC TGG GTG CTG GTG GGC GGA GTC CTT GCA GCT CTG GCC Val Thr Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala 260 265 270	815
45	GCA TAT CGC CTG ACA ACA GGC AGC GTG GTC ATC GTG GGT AGG ATC ATC Ala Tyr Arg Leu Thr Thr Gly Ser Val Val Ile Val Gly Arg Ile Ile 275 280 285	863
50	TTG TCT GGG AGG CCG GCT GTC ATT CCC GAC AGG GAA GTC CTT TAC CGG Leu Ser Gly Arg Pro Ala Val Ile Pro Asp Arg Glu Val Leu Tyr Arg 290 295 300	911

SEQ ID NO:9

SEQUENCE LENGTH:489 base pairs

SEQUENCE TYPE:nucleic acid

STRANDEDNESS:double

TOPOLOGY:linear

MOLECULE TYPE:cDNA to genomic RNA

CG ACA ACC GTG CCC CAA GAC GCG GTG TCG CGC TCA CAA CGG CGG GGT 47

Thr Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly

1 5 10 15

AGG ACA GGT AGG GGC AGG AGA GGC ATC TAC AGA TTT GTG ACT CCG GGA 95

Arg Thr Gly Arg Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro Gly

20 25 30

GAA CGG CCC TCG GGC ATG TTC GAT TCT TCG GTC CTG TGT GAG TGC TAT 143

Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr

35 40 45

GAC GCG GGC TGC GCT TGG ATC GAG CTC ACG CCC GCC GAG ACC TCA GTT 191

Asp Ala Gly Cys Ala Trp Ile Glu Leu Thr Pro Ala Glu Thr Ser Val

50 55 60

AGG TTG CGG GCT TAC CTA AAT ACA CCA GGG TTG CCC GTC TGC CAG GAC 239

Arg Leu Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp

65 70 75

CAC CTG GAA TTC TGG GAG AGC GTC TTC ACA GGC CTC ACC CAT ATA GAT 287

His Leu Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp

80 85 90 95

GCC CAC TTC TTG TCC CAG ACC AAG CAG GCA GGA GAC AAC TTC CCC TAC 335

Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr

100 105 110

CTG GTA GCA TAC CAA GCT ACA GTG TGC GCC AGG GCC CAG GCT CCA CCA 383

Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro

115 120 125

CCA TCG TGG GAT CAA ATG TGG AAG TGT CTC ATA CGG CTG AAA CCT ACC 431

Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr

130 135 140

5 CTA CAC GGG CCA ACA CCC CTG TTG TAT AGG CTG GGA GCC GTC CAA AAT 479
 Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
 145 150 155

10 GAG GTC ACC C 489
 Glu Val Thr
 160

15 SEQ ID NO:10
 SEQUENCE LENGTH:1076 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 TOPOLOGY:linear
 20 MOLECULE TYPE:cDNA to genomic RNA

25 GT GGT CTC CTG GGT GCC ATC GTG GTC AGC CTA ACG GGC CGC GAC AAG 47
 Gly Leu Leu Gly Ala Ile Val Val Ser Leu Thr Gly Arg Asp Lys
 1 5 10 15

30 AAC CAG GTC GAG GGG GAG GTT CAG GTG GTC TCC ACC GCA ACG CAA TCT 95
 Asn Gln Val Glu Gly Glu Val Gln Val Val Ser Thr Ala Thr Gln Ser
 20 25 30

35 TTC CTG GCG ACC TGC GTC AAT GGC GTG TGT TGG ACC GTC TAC CAT GGC 143
 Phe Leu Ala Thr Cys Val Asn Gly Val Cys Trp Thr Val Tyr His Gly
 35 40 45

40 GCC GGC TCG AAA ACC CTG GCC GGC CCG AAG GGT CCA GTC ACC CAA ATG 191
 Ala Gly Ser Lys Thr Leu Ala Gly Pro Lys Gly Pro Val Thr Gln Met
 50 55 60

45 TAC ACT AAT GTG GAC CAG GAC CTC GTC GGC TGG CCG GCG CCC TCC GGC 239
 Tyr Thr Asn Val Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Ser Gly
 65 70 75

50 GCG CGG TCC TTG ACA CCA TGC ACC TGC GGC AGC TCG GAC CTT TAC TTG 287
 Ala Arg Ser Leu Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu
 80 85 90 95

55 GTC ACG AGG CAT GCT GAT GTC ATT CCG GTG CGC CGG CGG GGC GAT AGC 335
 Val Thr Arg His Ala Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser
 100 105 110

5	AGG GGG AGC CTG CTT TCC CCC AGG CCC CTC TCC TAC TTG AAG GGC TCC Arg Gly Ser Leu Leu Ser Pro Arg Pro Leu Ser Tyr Leu Lys Gly Ser 115 120 125	383
10	TCA GGT GGT CCA CTG CTT TGC CCC TCG GGG CAC ATT GTG GGC ATC TTC Ser Gly Gly Pro Leu Leu Cys Pro Ser Gly His Ile Val Gly Ile Phe 130 135 140	431
15	CGG GCT GCC GTG TGC ACC CGG GGG GTT GCG AAG GCG GTG GAC TTT GTA Arg Ala Ala Val Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Val 145 150 155	479
20	CCT GTC GAG TCT ATG GAA ACT ACT ATG CGG TCT CCG GTC TTC ACG GAT Pro Val Glu Ser Met Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp 160 165 170 175	527
25	AAT TCA TCC CCC CCG GCC GTA CCG CAG ACA TTC CAA GTG GCC CAT CTG Asn Ser Ser Pro Pro Ala Val Pro Gln Thr Phe Gln Val Ala His Leu 180 185 190	575
30	CAT GCC CCC ACT GGC AGC GGC AAG AGC ACT AAG GTG CCG GCT GCA TAC His Ala Pro Thr Gly Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr 195 200 205	623
35	GCA GCC CAG GGA TAC AAG GTA CTC GTC CTG AAC CCG TCC GTT GCC GCC Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala 210 215 220	671
40	ACC TTA GGT TTT GGA GCA TAT ATG TCC AAG GCA CAT GGT GTC GAC CCT Thr Leu Gly Phe Gly Ala Tyr Met Ser Lys Ala His Gly Val Asp Pro 225 230 235	719
45	AAC ATC AGG ACT GGG GTA AGG ACC ATC ACT ACG GGC GCC CCC ATT ACA Asn Ile Arg Thr Gly Val Arg Thr Ile Thr Thr Gly Ala Pro Ile Thr 240 245 250 255	767
50	TAC TCC ACC TAT GGC AAG TTT CTT GCC GAC GGT GGT TGC TCC GGG GGC Tyr Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly 260 265 270	815
55	GCC TAT GAC ATC ATA ATA TGT GAT GAG TGC CAC TCA ACT GAC TCG ACT Ala Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ser Thr 275 280 285	863

5 TCC ATT TTG GGC ATT GGC ACG GTC CTG GAC CAA GCG GAG ACG GCT GGA 911
 Ser Ile Leu Gly Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly
 290 295 300

10 GCG CGG CTC GTC GTG CTC GCC ACC GCT ACG CCT CCA GGA TCG GTC ACT 959
 Ala Arg Leu Val Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr
 305 310 315

15 GTG CCT CAT CCC AAC ATC GAG GAG GTG GCC TTG TCC ACC ACT GGA GAG 1007
 Val Pro His Pro Asn Ile Glu Glu Val Ala Leu Ser Ser Thr Gly Glu
 320 325 330 335

20 ATT CCC TTC TAT GGC AAA GCC ATC CCC ATT GAG ACC ATC AAG GGG GGA 1055
 Ile Pro Phe Tyr Gly Lys Ala Ile Pro Ile Glu Thr Ile Lys Gly Gly
 340 345 350

25 AGG CAT CTC ATT TTC TGC CAC 1076
 Arg His Leu Ile Phe Cys His
 355
 Sequence Listing

30 SEQ ID NO:11
 SEQUENCE LENGTH:284 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 35 TOPOLOGY:linear
 MOLECULE TYPE:cDNA to genomic RNA

40 GTC GAC CCC AAT ATT AGA ACT GGG GTA AGG ACC ATC ACC ACG GGC GCT 48
 Val Asp Pro Asn Ile Arg Thr Gly Val Arg Thr Ile Thr Thr Gly Ala
 1 5 10 15

45 CCC ATT ACG TAT TCT ACC TAT GGC AAA TTC CTT GCC GAC GGT GGT TGC 96
 Pro Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly Cys
 20 25 30

50 TCT GGG GGC GCC TAT GAC ATC ATA ATC TGT GAT GAG TGC CAC TCA ACT 144
 Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ser Thr
 35 40 45

55 GAC TCG ACT TCC ATC TTG GGT ATC GGC ACA GCC CTG GAC CAA GCG GAG 192
 Asp Ser Thr Ser Ile Leu Gly Ile Gly Thr Ala Leu Asp Gln Ala Glu
 50 55 60

ACG GCT GGA GCA CGG CTT GTC GTG CTC GCC ACC GCT ACG CCT CCA GGG 240
 Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr Ala Thr Pro Pro Gly
 5 65 70 75 80

TCG GTC ACC GTG CCG CAT CCC AAC ATC GAG GAG GTA GCC TTG CC 284
 Ser Val Thr Val Pro His Pro Asn Ile Glu Glu Val Ala Leu
 10 85 90

SEQ ID NO:12
 SEQUENCE LENGTH:641 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 TOPOLOGY:linear
 MOLECULE TYPE:cDNA to genomic RNA

G GAC AAC TCA TCT CCC CCG GCG GTA CCG CAG ACA TTC CAG GTG GCC CAT 49
 Asp Asn Ser Ser Pro Pro Ala Val Pro Gln Thr Phe Gln Val Ala His
 25 1 5 10 15

CTA CAC GCT CCC ACT GGC AGC GGC AAG AGC ACT AAG GTG CCG GCT GCA 97
 Leu His Ala Pro Thr Gly Ser Gly Lys Ser Thr Lys Val Pro Ala Ala
 30 20 25 30

TAT GCA GCC CAA GGG TAC AAA GTA CTC GTC CTG AAC CCG TCC GTT GCC 145
 Tyr Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala
 35 35 40 45

GCC ACC TTA AGT TTC GGG GCG TAT ATG TCC AAG GCA CAT GGT GTT GAC 193
 Ala Thr Leu Ser Phe Gly Ala Tyr Met Ser Lys Ala His Gly Val Asp
 40 50 55 60

CCT AAT ATC AGA ACT GGG ACA AGG ACC ATC ACC ACG GGC GCT CCC ATC 241
 Pro Asn Ile Arg Thr Gly Thr Arg Thr Ile Thr Thr Gly Ala Pro Ile
 45 65 70 75 80

ACG TAC TCC ACC TAT GGC AAG TTC CTT GCA GAC GGT GGT TGC TCC GGA 289
 Thr Tyr Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly
 50 85 90 95

GGC GCC TAT GAC ATC ATA ATA TGC GAT GAG TGC CAC TCA ACA GAC TCG 337
 Gly Ala Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ser
 55 100 105 110

ACT TCC ATC TTA GGC ATT GGT ACG GTC CTG GAC CAA GCG GAG ACG GCT 385
 Thr Ser Ile Leu Gly Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala
 5 115 120 125

GGA GCG CGA CTC GTC GTG CTC GCC ACC GCT ACG CCT CCA GGA TCG GTC 433
 Gly Ala Arg Leu Val Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val
 10 130 135 140

ACT GTG CCA CAT CCC AAC ATC GAG GAG GTG GCC CTG TCC AAC ACT GGA 481
 Thr Val Pro His Pro Asn Ile Glu Glu Val Ala Leu Ser Asn Thr Gly
 15 145 150 155 160

GAG ATT CCC TTC TAT GGC AAA GCC ATC CCC ATT GAG GCC ATC AAG GGG 529
 Glu Ile Pro Phe Tyr Gly Lys Ala Ile Pro Ile Glu Ala Ile Lys Gly
 20 165 170 175

GGG AGG CAT CTC ATT TTC TGC CAT TCT AAG AAG AAG TGT GAT GAG CTC 577
 Gly Arg His Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu
 25 180 185 190

GCC ACG AAG CTG TCG GCC CTC GGA CTC AAT GCT GTA GCG TAC TAC CGG 625
 Ala Thr Lys Leu Ser Ala Leu Gly Leu Asn Ala Val Ala Tyr Tyr Arg
 30 195 200 205

GGT CTT GAT GTG TCC G 641
 Gly Leu Asp Val Ser
 35 210

SEQ ID NO:13
 40 SEQUENCE LENGTH:432 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 TOPOLOGY:linear
 45 MOLECULE TYPE:cDNA to genomic RNA

CA GGC GAG AGG CCG ACA GGG ATG TTT GAC AGC GTA GTG CTC TGT GAG 47
 Gly Glu Arg Pro Thr Gly Met Phe Asp Ser Val Val Leu Cys Glu
 50 1 5 10 15

TGC TAT GAT GCC GGG GCC GCC TGG TAC GAG CTT ACG CCT GCT GAG ACT 95
 Cys Tyr Asp Ala Gly Ala Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr
 55 20 25 30

5 ACG GTG AGA CTC CGG GCT TAT TTC AAC ACG CCC GGT TTG CCT GTA TGT 143
 Thr Val Arg Leu Arg Ala Tyr Phe Asn Thr Pro Gly Leu Pro Val Cys
 35 40 45

10 CAA GAC CAC CTA GAG TTC TGG GAA GCG GTC TTC ACA GGT CTC ACA CAC 191
 Gln Asp His Leu Glu Phe Trp Glu Ala Val Phe Thr Gly Leu Thr His
 50 55 60

15 ATT GAT GCC CAC TTC CTC TCC CAG ACG AAG CAA GGA GGA GAC AAC TTT 239
 Ile Asp Ala His Phe Leu Ser Gln Thr Lys Gln Gly Gly Asp Asn Phe
 65 70 75

20 GCG TAT CTA ACG GCC TAC CAG GCC ACA GTA TGC GCC AGG GCA AAG GCC 287
 Ala Tyr Leu Thr Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Lys Ala
 80 85 90 95

25 CCC CCT CCT TCG TGG GAC GTG ATG TGG AAG TGT CTA ATC AGG CTC AAA 335
 Pro Pro Pro Ser Trp Asp Val Met Trp Lys Cys Leu Ile Arg Leu Lys
 100 105 110

30 CCT ACA TTG ACT GGT CCT ACC CCC CTC CTG TAC CGC TTG GGT GCC GTG 383
 Pro Thr Leu Thr Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val
 115 120 125

35 ACT AAC GAG GTT ACC CTG ACG CAC CCC GTG ACG AAA TAT ATC GCC ACG T 432
 Thr Asn Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Ala Thr
 130 135 140

40 SEQ ID NO:14
 SEQUENCE LENGTH:369 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 45 TOPOLOGY:linear
 MOLECULE TYPE:cDNA to genomic RNA

50 ATG GGC ACG AAT CCT AAA CCT CAA AGA AAA ACC AAA AGA AAC ACT AAC 48
 Met Gly Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
 1 5 10 15

55 CGT CGC CCA CAA GAC GTT AAG TTT CCG GGC GGC GGC CAG ATC GTT GGC 96
 Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly
 20 25 30

5	GGA GTA TAC TTG TTG CCG CGC AGG GGC CCC AGA TTG GGT GTG CGC GCG Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala	144
	35 40 45	
10	ACA AGG AAG ACT TCG AAG CGG TCC CAG CCA CGT GGG GGG CGC CGG CCC Thr Arg Lys Thr Ser Lys Arg Ser Gln Pro Arg Gly Gly Arg Arg Pro	192
	50 55 60	
15	ATC CCT AAA GAT CGG CGC TCC ACT GGC AAG TCC TGG GGG AAA CCA GGA Ile Pro Lys Asp Arg Arg Ser Thr Gly Lys Ser Trp Gly Lys Pro Gly	240
	65 70 75 80	
20	TAC CCC TGG CCC CTA TAT GGG AAT GAG GGA CTC GGC TGG GCA GGG TGG Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Leu Gly Trp Ala Gly Trp	288
	85 90 95	
25	CTT CTG TCC CCC CGA GGT TCC CGT CCC TCT TGG GGC CCC ACT GAC CCC Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro	336
	100 105 110	
30	CGG CAT AGG TCG CGC AAT GTG GGT AAG GTC ATC Arg His Arg Ser Arg Asn Val Gly Lys Val Ile	369
	115 120	

35

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45

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SEQ ID NO:15

SEQUENCE LENGTH:932 base pairs

SEQUENCE TYPE:nucleic acid

STRANDEDNESS:double

TOPOLOGY:linear

MOLECULE TYPE:cDNA to genomic RNA

CG CGC AAC TTG GGT AAG GTC ATC GAT ACC CTC ACA TGC GGC TTC GCC 47

Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala

1 5 10 15

GAC CTC ATG GGG TAC ATT CCG CTT GTC GGC GCC CCC CTA GGG GGT GCT 95

Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala

20 25 30

GCC AGG GCC CTG GCA CAT GGT GTC CGG GTT CTG GAG GAC GGC GTG AAC 143

Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn

35 40 45

TAT GCA ACA GGG AAT TTG CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG 191

Tyr Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu

50 55 60

GCT TTG CTG TCC TGT TTG ACC ATC CCA GCT TCC GCT TAT GAG GTG CGC 239

Ala Leu Leu Ser Cys Leu Thr Ile Pro Ala Ser Ala Tyr Glu Val Arg

65 70 75

AAC GTA TCC GGG ATA TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGT 287

Asn Val Ser Gly Ile Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser

80 85 90 95

ATT GTG TAT GAG GCA GCG GAC ATG ATC ATG CAT ACC CCC GGG TGC GTG 335

Ile Val Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val

100 105 110

CCC TGC GTT CGG GAG AAC AAC TCC TCC CGT TGC TGG GCA GCG CTC ACT 383

Pro Cys Val Arg Gly Asn Asn Ser Ser Arg Cys Trp Ala Ala Leu Thr

115 120 125

CCC ACG TTA GCG GCC AGG AAC ACC AGC GTC CCC ACT ACG ACA ATA CGA 431

Pro Thr Leu Ala Ala Arg Asn Thr Ser Val Pro Thr Thr Thr Ile Arg

130 135 140

5	CGG CAT GTC GAT TTG CTC GTT GGG GCG GCT GCT TTC TGC TCC GCT ATG Arg His Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met 145 150 155	479
10	TAC GTG GGG GAT CTC TGT GGA TCT GTC TTC CTC GTT TCC CAG CTG TTC Tyr Val Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe 160 165 170 175	527
15	ACT TTC TCA CCT CGT CGG CAT GAG ACA GTA CAG GAC TGC AAC TGC TCA Thr Phe Ser Pro Arg Arg His Gln Thr Val Gln Asp Cys Asn Cys Ser 180 185 190	575
20	ATC TAT CCC GGC CAC TTG ACA GGT CAT CGC ATG GCT TGG GAT ATG ATG Ile Tyr Pro Gly His Leu Thr Gly His Arg Met Ala Trp Asp Met Met 195 200 205	623
25	ATG AAC TGG TCA CCT ACA ACA GCC CTA GTG GTG TCG CAT CTA CTC CGG Met Asn Trp Ser Pro Thr Thr Ala Leu Val Val Ser His Leu Leu Arg 210 215 220	671
30	ATC CCA CAA GCT GTC ATG GAC ATG GTG GCG GGG GCT CAC TGG GGA GTC Ile Pro Gln Ala Val Met Asp Met Val Ala Gly Ala His Trp Gly Val 225 230 235	719
35	CTA GCG GGC CTC GCC TAC TAT TCC ATG GTG GGG AAC TGG GCT AAG GTT Leu Ala Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp Ala Lys Val 240 245 250 255	767
40	TTG ATT GTG ATG CTA CTC TTC GCC GGC GTT GAC GGG ACC ACC TAT GTG Leu Ile Val Met Leu Leu Phe Ala Gly Val Asp Gly Thr Thr Tyr Val 260 265 270	815
45	ACA GGG GGG ACG ACA GGC CGC ACC ACC AGC TCG TTC GCA TCC CTC TTT Thr Gly Gly Thr Thr Gly Arg Thr Thr Ser Ser Phe Ala Ser Leu Phe 275 280 285	863
50	ACA CTT GGG TCG CAT CAG AAG GTC CAG CTT ATA AAT ACC AAT GGC AGC Thr Leu Gly Ser His Gln Lys Val Gln Leu Ile Asn Thr Asp Gly Ser 290 295 300	911
55	TGG CAC ATC AAC AGG ACC GCC Trp His Ile Asn Arg Thr Ala 305 310	932

SEQ ID NO:16
 SEQUENCE LENGTH:559 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 TOPOLOGY:linear
 MOLECULE TYPE:cDNA to genomic RNA

5	CGC CGG TAT GAG ACG GCG CAA GAC TGC AAT TGC TCA CTC TAT CCC GGT	48
	Arg Arg Tyr Glu Thr Ala Gln Asp Cys Asn Cys Ser Leu Tyr Pro Gly	
	1 5 10 15	
15	CAC GTA TCT GGT CAC CGC ATG GCT TGG GAT ATG ATG ATG AAC TGG TCA	96
	His Val Ser Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp Ser	
	20 25 30	
20	CCT ACA ACG GCC CTA GTG GTA TCG CAG CTA CTC CGG ATC CCA CAA GCC	144
	Pro Thr Thr Ala Leu Val Val Ser Gln Leu Leu Arg Ile Pro Gln Ala	
	35 40 45	
25	GTC GTG GAC ATG GTG GCG GGG GCC CAC TGG GGA GTC CTA GCG GGC CTT	192
	Val Val Asp Met Val Ala Gly Ala His Trp Gly Val Leu Ala Gly Leu	
	50 55 60	
30	GCC TAC TAT TCC ATG GTG GCG AAC TGG GCT AAG GTC TTG GTT GTG ATG	240
	Ala Tyr Tyr Ser Met Val Ala Asn Trp Ala Lys Val Leu Val Val Met	
	65 70 75 80	
35	CTA CTC TTT GCC GGC GTT GAC GAC GGG AAG ACC ACC GTG ACG GGG GGG	288
	Leu Leu Phe Ala Gly Val Asp Asp Gly Lys Thr Thr Val Thr Gly Gly	
	85 90 95	
40	AGC GCA GCC TTC CAG TCC AGG AAG TTA GTG TCC TTC TTC TCA CCA GGG	336
	Ser Ala Ala Phe Gln Ser Arg Lys Leu Val Ser Phe Phe Ser Pro Gly	
	100 105 110	
45	CCG AAA CAA AAT ATC CAG CTT GAT AAC ACC AAC GGC AGC TGG CAC ATC	384
	Pro Lys Gln Asn Ile Gln Leu Asp Asn Thr Asn Gly Ser Trp His Ile	
	115 120 125	
50	AAC AGG ACT GCC CTG AAT TGC AAT GAC TCC CTC CAA ACT GGG TTC ATC	432
	Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser Leu Gln Thr Gly Phe Ile	
	130 135 140	
55		

5 GCT GCG CTG TTC TAC GCG CAC AAG TTC AAT TCG TCC GGA TGC CTA GAG 480
 Ala Ala Leu Phe Tyr Ala His Lys Phe Asn Ser Ser Gly Cys Leu Glu
 145 150 155 160

10 CGC ATG GCC AGC TGC CGC CCC ATT GAC AAG TTC GCG CAG GGG TGG GGT 528
 Arg Met Ala Ser Cys Arg Pro Ile Asp Lys Phe Ala Gln Gly Trp Gly
 165 170 175

15 CCC ATC ACT CAC GAT ACG CCT AAG ATC CCG G 559
 Pro Ile Thr His Asp Thr Pro Lys Ile Pro
 180 185

20 SEQ ID NO:17
 SEQUENCE LENGTH:276 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:double
 25 TOPOLOGY:linear
 MOLECULE TYPE:cDNA to genomic RNA

30 GA CAC CGT ATG GCA TGG GAC ATG ATG ATG AAC TGG TCG CCC ACG GCT 47
 His Arg Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr Ala
 1 5 10 15

35 ACC ATG ATT CTG GCG TAT GTG ATG CGC ATC CCC GAG GTC GTC ATG GAC 95
 Thr Met Ile Leu Ala Tyr Val Met Arg Ile Pro Glu Val Val Met Asp
 20 25 30

40 ATC ATT GGC GGC GCT CAC TGG GGC GTC ATG TTC GGC TTG GGC TAT TTT 143
 Ile Ile Gly Gly Ala His Trp Gly Val Met Phe Gly Leu Gly Tyr Phe
 35 40 45

45 TCT ATG CAG GGC GCT TGG GCA AAA GTC GTT GTC ATC CTT CTG CTG GCC 191
 Ser Met Gln Gly Ala Trp Ala Lys Val Val Val Ile Leu Leu Leu Ala
 50 55 60

50 GCT GGG GTG GAT GCG ACT ACC CTC AGC GTT GGG GGC TCT GCC GCG CAC 239
 Ala Gly Val Asp Ala Thr Thr Leu Ser Val Gly Gly Ser Ala Ala His
 65 70 75

55 ACC ACC GGC GGC CTT GTC GGC TTG TTC AAG CCT GGC G 276
 Thr Thr Gly Gly Leu Val Gly Leu Phe Lys Pro Gly
 80 85 90

SEQ ID NO:18

SEQUENCE LENGTH:742 base pairs

SEQUENCE TYPE:nucleic acid

STRANDEDNESS:double

TOPOLOGY:linear

MOLECULE TYPE:cDNA to genomic RNA

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10 CG CTT GTC GGC GCC CCC CTA GGG GGT GCT GCC AGG GCC CTG GCA CAT      47
    Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg Ala Leu Ala His
      1             5             10             15

15 GGT GTC CGG GTT CTG GAG GAC GGC GTG AAC TAT GCA ACA GGG AAT TTG      95
    Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala Thr Gly Asn Leu
                20             25             30

20 CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTG CTG TCC TGT TTG      143
    Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu Leu Ser Cys Leu
                35             40             45

25 ACC ATC CCA GCT TCC GCT TAT GAG GTG CGC AAC GTA TCC GGG ATA TAC      191
    Thr Ile Pro Ala Ser Ala Tyr Glu Val Arg Asn Val Ser Gly Ile Tyr
                50             55             60

30 CAT GTC ACG AAC GAC TGC TCC AAC TCA AGT ATT GTG TAT GAG GCA GCG      239
    His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val Tyr Glu Ala Ala
        65             70             75

35 GAC ATG ATC ATG CAT ACC CCC GGG TGC GTG CCC TGC GTT CGG GAG AAC      287
    Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys Val Arg Glu Asn
        80             85             90             95

40 AAC TCC TCC CGT TGC TGG GCA GCG CTC ACT CCC ACG TTA GCG GCC AGG      335
    Asn Ser Ser Arg Cys Trp Ala Ala Leu Thr Pro Thr Leu Ala Ala Arg
                100             105             110

45 AAC ACC AGC GTC CCC ACT ACG ACA ATA CGA CGG CAT GTC GAT TTG CTC      383
    Asn Thr Ser Val Pro Thr Thr Thr Ile Arg Arg His Val Asp Leu Leu
                115             120             125

50 GTT GGG GCG GCT GCT TTC TGC TCC GCT ATG TAC GTG GGG GAT CTC TGT      431
    Val Gly Ala Ala Ala Phe Cys Ser Ala Met Tyr Val Gly Asp Leu Cys
                130             135             140
55

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5 GGA TCT GTC TTC CTC GTT TCC CAG CTG TTC ACT TTC TCA CCT CGT CGG 479
 Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Phe Ser Pro Arg Arg
 145 150 155

10 CAT GAG ACA GTA CAG GAC TGC AAC TGC TCA ATC TAT CCC GGC CAC TTG 527
 His Glu Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His Leu
 160 165 170 175

15 ACA GGT CAT CGC ATG GCT TGG GAT ATG ATG ATG AAC TGG TCA CCT ACA 575
 Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr
 180 185 190

20 ACA GCC CTA GTG GTG TCG CAT CTA CTC CGG ATC CCA CAA GCT GTC ATG 623
 Thr Ala Leu Val Val Ser His Leu Leu Arg Ile Pro Gln Ala Val Met
 195 200 205

25 GAC ATG GTG GCG GGG GCC CAC TGG GGA GTC CTA GCG GGC CTT GCC TAC 671
 Asp Met Val Ala Gly Ala His Trp Gly Val Leu Ala Gly Leu Ala Tyr
 210 215 220

30 TAT TCC ATG GTG GGG AAC TGG GCT AAG GTT TTG ATT GTG ATG CTA CTC 719
 Tyr Ser Met Val Gly Asn Trp Ala Lys Val Leu Ile Val Met Leu Leu
 225 230 235

35 TTC GCC GGC GTT GAC GGG ACC AC 742
 Phe Ala Gly Val Asp Gly Thr
 240 245

40 SEQ ID NO:19
 SEQUENCE LENGTH:20 base pairs
 SEQUENCE TYPE:nucleic acid
 STRANDEDNESS:single
 TOPOLOGY:linear

45 GGATACACCG GTGACTTTGA 20

50 SEQ ID NO:20
 SEQUENCE LENGTH:20 base pairs
 SEQUENCE TYPE:nucleic acid
 55 STRANDEDNESS:single
 TOPOLOGY:linear

TGCATGCACG TGGCGATGTA
Sequence Listing

20

5

SEQ ID NO:21
SEQUENCE LENGTH:20 base pairs
SEQUENCE TYPE:nucleic acid
STRANDEDNESS:single
TOPOLOGY:linear

10

15 GATGCCCCACT TCCTCTCCCA

20

SEQ ID NO:22
SEQUENCE LENGTH:20 base pairs
SEQUENCE TYPE:nucleic acid
STRANDEDNESS:single
TOPOLOGY:linear

20

25

GTCAGGGTAA CCTCGTTGGT

20

30

Claims

(1) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 1.

35

(2) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 2.

40

(3) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 3.

(4) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 4.

45

(5) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 5.

(6) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 6.

50

(7) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 7.

55

(8) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 8.

(9) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the

whole or a part of the amino acid sequence represented by the SEQ ID NO. 9.

(10) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 10.

5 (11) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 11.

(12) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 12.

(13) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a non-structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 13.

15 (14) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 14.

(15) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 15.

20 (16) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 16.

(17) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 17.

25 (18) A DNA fragment comprising a base sequence which encodes a non-A non-B hepatitis-specific antigen polypeptide from a structural protein of the non-A non-B hepatitis virus, said polypeptide consisting of the whole or a part of the amino acid sequence represented by the SEQ ID NO. 18.

(19) An expression vector containing the DNA fragment according to any one of claims 1 to 18 in a cloning site downstream of a promoter gene in the vector.

(20) An expression vector according to claim 19, wherein the vector is a plasmid.

(21) An expression vector according to claim 20, wherein the vector is plasmid Trp-TrpE-C11-C21.

(22) An expression vector according to claim 20, wherein the vector is plasmid Trp-TrpE-C11-7.

35 (23) A transformant comprising a host cell transformed with the expression vector according to any one of claims 19 to 22

(24) A transformant according to claim 23, wherein the host cell is *Escherichia coli*.

(25) A process for producing a recombinant non-A non-B hepatitis-specific antigen polypeptide obtained by expression of the DNA fragment according to any one of claims 1 to 18, which comprises the following steps of:

40 constructing a replicable expression vector which is capable of expressing said DNA fragment in an appropriate host cell;

obtaining a transformant by introducing said expression vector into the host cell;

producing said recombinant polypeptide by culturing said transformant under such conditions that said DNA fragment is expressed; and

45 recovering the recombinant polypeptide.

(26) A recombinant non-A non-B hepatitis-specific antigen polypeptide obtained by expressing the DNA fragment according to any one of claims 1 to 18.

(27) A method for amplifying a non-A non-B hepatitis virus gene using sense and/or antisense sequence synthesized on the basis of a partial base sequence of the DNA fragment according to any one of claims 1 to 18.

50 (28) A single strand DNA sequence for PCR primer represented by SEQ ID NO. 19.

(29) A single strand DNA sequence for PCR primer represented by SEQ ID NO. 20.

(30) A single strand DNA sequence for PCR primer represented by SEQ ID NO. 21.

(31) A single strand DNA sequence for PCR primer represented by SEQ ID NO. 22.

55 (32) A method for detecting a non-A non-B hepatitis virus gene in a fluid sample to be examined, which comprises the following steps of:

isolating RNA from a fluid sample to be examined;

synthesizing cDNA by treating the RNA with a reverse transcriptase;

subjecting the cDNA to polymerase chain reaction using at least one single strand DNA sequence according to any one of claims 28 to 31; and

detecting an amplified non-A non-B hepatitis virus gene.

(33) An immunological method for detecting an antibody directed against a non-A non-B hepatitis virus antigen, which comprises the following steps of:

incubating a fluid sample which may contain an anti-non-A non-B hepatitis virus antibody, in the presence of at least one recombinant non-A non-B hepatitis-specific-antigen polypeptide according to claim 26 under such conditions that said antibody and said polypeptide are capable of undergoing antigen-antibody reaction; and

detecting an antigen-antibody complex.

(34) Use of the recombinant non-A non-B hepatitis-specific antigen polypeptide according to claim 26 in the detection of the non-A non-B hepatitis virus.

(35) Use of the single strand DNA sequence for PCR primer according to any one of claims 28 to 31 in the detection of the non-A non-B hepatitis virus.

Fig. 1a

```

10      20      30      40      50      60
CGCAGTCATTCCAAGTGGCCCATCTACACGCTCCCACTGGCAGCGGCAAGAGTACTAAAG
  GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal

70      80      90     100     110     120
TGCCGGCTGCATATGCCAGCCCAAGGTACAAAGGTGCTCGTCTCAACCCGTCCTGTCGCG
  ProAlaAlaTyrAlaSerGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla

130     140     150     160     170     180
CCACCTTAGGTTTTGGAGCGTATATGTCTAAGGCACATGGCACCGACCCCAACATCAGAA
  ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyThrAspProAsnIleArgThr

190     200     210     220     230     240
CTGGGTAAGGACTATCACCCACAGGCGGCCCATCAGTACTCCACCTACGGCAAGTTCC
  GlyValArgThrIleThrThrGlyAlaProIleThrTyrSerThrTyrGlyLysPheLeu

250     260     270     280     290     300
TTGCCGACGGTGGTTGTTCTGGGGCGCTTATGACATCATATAATGTGTGATGAGTGCCACT
  AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleMetCysAspGluCysHisSer

310     320     330     340     350     360
CAACTGACGCGACTTCCATCTTGGGCATCGGCACGGTCTCGACCAAGCGGAGACGGCTG
  ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly

```


Fig. 1b

370 380 390 400 410 420
 GAGCAGGCTCGTCGCTCGCCACCGCTACGGCTCCGGGATCGGTACCGTGCCACACC
 AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro

430 440 450 460 470 480
 CGAATATTGAGGAGGTGGCCCTGTCTAACACTGGAGAGATCCCCCTTCTATGGCAAGCCA
 AsnIleGluGluValAlaLeuSerAsnThrGlyGluIleProPheTyrGlyLysGlyIle

490 500 510 520 530 540
 TCCCCATTGAAGTCATCAAGGGGGAAGGCATCTCATTTTCTGCCATTCCAAGAAGAAGT
 ProIleGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCys

550 560 570 580 590 600
 GCGACGAGCTCGCCGCGAAGTTGTACAGCCCTCGGGATTAAATGTGTGGCATACTACCGGG
 AspGluLeuAlaAlaLysLeuSerGlyLeuGlyIleAsnAlaValAlaTyrTyrArgGly

610 620 630 640 650 660
 GTCTTGATGTGTCGTCATACCGACCGGAGACGTCGTTGTCGTGGCAACAGACGCTC
 LeuAspValSerValIleProThrSerGlyAspValValValAlaThrAspAlaLeu

670 680 690 700 710 720
 TAATGACGGCTATACCGGCGATTGACTCAGTCGATCGACTGTAACACATGCGTCACCC
 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln

730 740 750 760
 AGACAGTCGACTTCAGCTTGGACCCACCTTCACCATTTGAGAC
 ThrValAspPheSerLeuAspProThrPheThrIleGlu

Fig. 2

10 20 30 40 50 60
 CACGCCCGTTTGCCCGTGTGTCAAGACCACCTGGAGTTCTGGGAAGCGGTCTTCACAGG
 ThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluAlaValPheThrGly

70 80 90 100 110 120
 TCTCACGCACATTGATGCCCACTTCCTCTCCAGACAAAGCAAGGAGGAGACAACCTTCGC
 LeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnGlyGlyAspAsnPheAla

130 140 150 160 170 180
 GTATCTAACGGCCTACCAGGCCACAGTGTGCGCTAGGGCAAAGGCCCTCCTCCCTCGTG
 TyrLeuThrAlaTyrGlnAlaThrValCysAlaArgAlaLysAlaProProProSerTrp

190 200 210 220 230 240
 GGATGTGATGTGGAAATGTCTAGCTAGGCTGAAGCCTACACTAATTGGTCCTACCCCCCT
 AspValMetTrpLysCysLeuAlaArgLeuLysProThrLeuIleGlyProThrProLeu

250 260 270 280 290 300
 CCTGTACCGCTTGGGTGCCGTGACCAACGAGGTTACCCTGACGCACCCCGTGACGAAATA
 LeuTyrArgLeuGlyAlaValThrAsnGluValThrLeuThrHisProValThrLysTyr

310 320 330 340 350 360
 CATCGCCACGTGCATGCAAGCTGACCTCGAGATCATGACGAGCACATGGGTCCTAGCAGG
 IleAlaThrCysMetGlnAlaAspLeuGluIleMetThrSerThrTrpValLeuAlaGly

370 380 390 400 410 420
 GGGGGTGTAGCCGCCGTGGCAGCTTACTGCCTGGCAACCGGCTGTGTTTCCATCATCGG
 GlyValLeuAlaAlaValAlaAlaTyrCysLeuAlaThrGlyCysValSerIleIleGly

430 440 450 460 470 480
 CCGCCTACACCTGAATGATCAAGTGGTTGTGACTCCTGACAAAGAAATCTTATATGAGGC
 ArgLeuHisLeuAsnAspGlnValValValThrProAspLysGluIleLeuTyrGluAla

490 500 510 520 530 540
 CTTTGATGAGATGGAAGAATGCGCCTCCAAAGCCGCCCTCATTGAGGAAGGGCAGCGGAT
 PheAspGluMetGluGluCysAlaSerLysAlaAlaLeuIleGluGluGlyGlnArgMet

550 560 570 580 590 600
 GGCGGAGATGCTCAAGTCTAAGATACAAGGCCTCCTACAACAGGCCACAAGACAGGCCCA
 AlaGluMetLeuLysSerLysIleGlnGlyLeuLeuGlnGlnAlaThrArgGlnAlaGln

610
 AGACATACAGCCAGC
 AspIleGlnPro

Fig. 3a

10 20 30 40 50 60
 GTGAGCGAGCCTCAGGAATGTTTGACAGTGTAGTGCTCTGTGAGTGTGCTATGACGCAGGGG
 GluArgAlaSerGlyMetPheAspSerValValLeuCysGluCysTyrAspAlaGlyAla

70 80 90 100 110 120
 CTGCATGGTACGAGCTTACACCAGCGGAGACCACCGTCAGGCTCAGAGCGTATTTCAACA
 AlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArgAlaTyrPheAsnThr

130 140 150 160 170 180
 CACCTGGCTTGCCTGTGTGTCAGACCATCTTGAGTTCTGGGAGGCAGTTTTCACCGGCC
 ProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluAlaValPheThrGlyLeu

190 200 210 220 230 240
 TCACACACATAGATGCCCACTTCCTTTCCAGACAAAGCAAGCAGGGGACAATTTTCGCAT
 ThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnAlaGlyAspAsnPheAlaTyr

250 260 270 280 290 300
 ACTTGACAGCCTACCAGGCTACAGTGTGGCCAGAGCCAAAGCCCTCCCCCGTCCCTGGG
 LeuThrAlaTyrGlnAlaThrValCysAlaArgAlaLysAlaProProProSerTrpAsp

310 320 330 340 350 360
 ACGTCATGTGGAAGTGCCCTGACTCGGCTCAAGCCCCACGCTTGTGGCCCTACACCCCTTC
 ValMetTrpLysCysLeuThrArgLeuLysProThrLeuValAlaProThrProLeuLeu

Fig. 3b

370 380 390 400 410 420
 TGTACCGTTTAGGCTCTGTTACTAACGAGGTCACCTCACACATCCTGTGACGAAATACA
 TyrArgLeuGlySerValThrAsnGluValThrLeuThrHisProValThrLysTyrIle

430 440 450 460 470 480
 TCGCCACTTGCATGCAAGCTGACCTTGAGGTTCATGACCCAGCAGTGGGTCTCCTAGCTGGGG
 AlaThrCysMetGlnAlaAspLeuGluValMetThrSerThrTrpValLeuAlaGlyGly

490 500 510 520 530 540
 GGGTCTTGGCAGCCGTCGCCGGTATTGCCCTGGCGACTGGGTGTGTCTCCATCATCGGCC
 ValLeuAlaAlaValAlaAlaTyrCysLeuAlaThrGlyCysValSerIleIleGlyArg

550 560 570 580 590 600
 GCTTGACATCAATCAGCGAGCCGTCGTTCGACCAGACAAGGAGGTCTCTTTATGAGGCTT
 LeuHisIleAsnGlnArgAlaValValAlaProAspLysGluValLeuTyrGluAlaPhe

610 620 630 640 650 660
 TTGATGAGATGGAGGAGTGTGCCCTCTAAAGCGGCTCTCATTTGAAGAGGGGCAGCGGATAG
 AspGluMetGluGluCysAlaSerLysAlaAlaLeuIleGluGluGlyGlnArgIleAla

670 680 690 700 710 720
 CCGAGATGCTGAAGTCCAAGATCCAAGGCTTATTGCAGCAAGCCCTCTAAACAGGCCCCAGG
 GluMetLeuLysSerLysIleGlnGlyLeuLeuGlnGlnAlaSerLysGlnAlaGlnAsp

730 740 750 760 770
 ACATACAACCCGCTGTGCAGCCTCATGGCCCCAAGGTGGAGCAATTCTTGGGC
 IleGlnProAlaValGlnProHisGlyProArgTrpSerAsnSerGly

Fig. 4

10 20 30 40 50 60
 CTGGTATGAACTTACGCCTGCTGAGACTACGGTGAGACTCCGGGCCTATTTCAACACGCC
 TrpTyrGluLeuThrProAlaGluThrThrValArgLeuArgAlaTyrPheAsnThrPro

70 80 90 100 110 120
 CGGCCTGCCTGTGTGTCAAGACCACCTGGAATTCGAGGAGCGGTCTTCACAGGTCTCAC
 GlyLeuProValCysGlnAspHisLeuGluPheTrpGluAlaValPheThrGlyLeuThr

130 140 150 160 170 180
 ACACATCGATGCCCACTTCCTCTCCAGACGAAGCAAGGAGGAGATAACTTTGCATATTT
 HisIleAspAlaHisPheLeuSerGlnThrLysGlnGlyGlyAspAsnPheAlaTyrLeu

190 200 210 220 230 240
 AACAGCCTACCAGGCCACAGTCTGCGCTAGGGCAAAGGCTCCCCCTCCTTCGTGGGACGT
 ThrAlaTyrGlnAlaThrValCysAlaArgAlaLysAlaProProProSerTrpAspVal

250 260 270 280 290 300
 GATGTGGAAGTGTGTTGATTAGGCTCAAACCTACACTGACTGGTCCTACCCCCCTCCTGTA
 MetTrpLysCysLeuIleArgLeuLysProThrLeuThrGlyProThrProLeuLeuTyr

310 320 330 340 350 360
 CCGCTTGGGTGCCGTGACCAACGAGGTTACCCTGACTCACCCCATGACGAAATATATCGC
 ArgLeuGlyAlaValThrAsnGluValThrLeuThrHisProMetThrLysTyrIleAla

370 380 390 400 410 420
 CACTTGATGCAAGCTGATCTTGAGATCATGACAAGCACATGGGTCTTGGCGGGGGGGT
 ThrCysMetGlnAlaAspLeuGluIleMetThrSerThrTrpValLeuAlaGlyGlyVal

430 440 450 460 470 480
 GCTAGCCGCTGTGGCAGCTTACTGCCTAGCGACCGGCTGCATTTCCATCATTGGCCGCCT
 LeuAlaAlaValAlaAlaTyrCysLeuAlaThrGlyCysIleSerIleIleGlyArgLeu

490 500 510 520 530 540
 TCACCTGAATGATCGGGTGGTTCGTGACCCCTGATAAGGAAATTTATATGAGGCCTTTGA
 HisLeuAsnAspArgValValValThrProAspLysGluIleLeuTyrGluAlaPheAsp

550 560 570 580 590 600
 TGAGATGGAAGAGTGCGCCTCCAAAGCCGCCCTCATTGAGGAAGGGCAGCGGATGGCGGA
 GluMetGluGluCysAlaSerLysAlaAlaLeuIleGluGluGlyGlnArgMetAlaGlu

610 620 630
 GATGCTGAAGTCTAAAATACAAGGCCTCTT
 MetLeuLysSerLysIleGlnGlyLeu

Fig. 5a

10 20 30 40 50 60
 GGGATCAACCCTAACATCAGGACCGGAGTACGGACCGTGACACCGGGGACTCCATCACC
 GlyIleAsnProAsnIleArgThrGlyValArgThrValThrThrGlyAspSerIleThr

70 80 90 100 110 120
 TACTCCACTTATGGCAAGTTTATCGCAGATGGAGGTTCGCCACCGTGGTGCCTATGACGTC
 TyrSerThrTyrGlyLysPheIleAlaAspGlyGlyCysAlaArgGlyAlaTyrAspVal

130 140 150 160 170 180
 ATCATATGCGACGAATGCCATTTCAGTGGACGCTACTACCATCCTTGGCATTGGAAACAGTC
 IleIleCysAspGluCysHisSerValAspAlaThrThrIleLeuGlyIleGlyThrVal

190 200 210 220 230 240
 CTTGACCGGCTGAGACCGCAGGTGCCAGGCTAGTGGTTTTAGCCACAGCCACGCCACCC
 LeuAspGlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrProPro

250 260 270 280 290 300
 GGTACGGTAACAACCTCCCCACGCTAACATAGAGGAGGTGGCCCTTGGTCACGAAGCGGAG
 GlyThrValThrThrProHisAlaAsnIleGluGluValAlaLeuGlyHisGluGlyGlu

310 320 330 340 350 360
 ATTCCCTTTTATGGCAAGGCTATTCCTCCCTAGCTTTCATCAAGGGGGGCGACACCTAATT
 IleProPheTyrGlyLysAlaIleProLeuAlaPheIleLysGlyGlyArgHisLeuIle

Fig. 5b

370 380 390 400 410 420
 TTTTGCCATTCAAAGAAGTGGCAGAGCTCGCAGCAGCCCTTCGGGGCATGGGTATC
 PheCysHisSerLysLysCysAspGluLeuAlaAlaLeuArgGlyMetGlyIle

430 440 450 460 470 480
 AATGCCGTTGCCCTACTACAGGGGTCTCGACGTCICCGTTATACCAACTCAAGGAGACGTG
 AsnAlaValAlaTyrTyrArgGlyLeuAspValSerValIleProThrGlnGlyAspVal

490 500 510 520 530 540
 GTGGTTGTCGCCACCGATGCCCTAATGACTGGATACACCGGTGACTTTGACTCTGTGCATC
 ValValValAlaThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSerValIle

550 560 570 580 590 600
 GACTGCAACGTTGCACCTCAGATTGTTGACTTTAGCCTAGACCCAACTTTTACCATC
 AspCysAsnValAlaValThrGlnIleValAspPheSerLeuAspProThrPheThrIle

610 620 630 640 650 660
 ACCACTCAAACCGTCCCTCAGGAGGCTGTCTCCCGTAGTCAACGTAGAGGGAGAACTGGG
 ThrThrGlnThrValProGlnGluAlaValSerArgSerGlnArgArgGlyArgThrGly

670 680 690 700 710 720
 AGGGGGCGACTGGGCACTTACAGGTATGTCTCTCAGCGGAGAGGCCGTCTGGGATGTTT
 ArgGlyArgLeuGlyThrTyrArgTyrValSerSerGlyGluArgProSerGlyMetPhe

730 740 750 760 770 780
 GACAGCGTAGTACTCTGCGAGTGTATGATGCCGGGGCAGCCTGGTACGAGCTTACACCT
 AspSerValValLeuCysGluCysTyrAspAlaGlyAlaAlaTrpTyrGluLeuThrPro

Fig. 5c

790 800 810 820 830 840
 GCTGAGACCACAGTGAGACTCCGGGCTTATTTCAACACGCCCGGTTTGGCCGTGTGTCAA
 AlaGluThrThrValArgLeuArgAlaTyrPheAsnThrProGlyLeuProValCysGln

850 860 870 880 890 900
 GACCACCTGGAGTTCTGGGAAGCGGTCTTCACAGGTCTCACGCACATTGATGCCCACTTC
 AspHisLeuGluPheTrpGluAlaValPheThrGlyLeuThrHisIleAspAlaHisPhe

910 920 930 940 950 960
 CTCTCCAGACAAAGCAAGGAGGAGACAACCTTCGCGTATCTAACGGCCTACCAGGCCACA
 LeuSerGlnThrLysGlnGlyGlyAspAsnPheAlaTyrLeuThrAlaTyrGlnAlaThr

970 980 990 1000 1010 1020
 GTGTGCGCTAGGGCAAAGGCCCTCCTCCCTCGTGGGATGTGATGTGGAAATGTCTAGCT
 ValCysAlaArgAlaLysAlaProProProSerTrpAspValMetTrpLysCysLeuAla

1030 1040 1050 1060 1070 1080
 AGGCTGAAGCCTAGACTAATTGGTCCTACCCCCCTCCTGTACCGCTTGGGTGCCGTGACC
 ArgLeuLysProThrLeuIleGlyProThrProLeuLeuTyrArgLeuGlyAlaValThr

1090 1100 1110 1120 1130 1140
 AACGAGGTTACCCTGACGCACCCCGTGACGAAATACATCGCCACGTGCATGCAAGTGAAC
 AsnGluValThrLeuThrHisProValThrLysTyrIleAlaThrCysMetGlnValAsn

1150 1160 1170 1180 1190 1200
 CTCGAGATCATGACGAGCACATGGGTCCTAGCAGGGGGGTGCTAGCCGCCGTGGCAGCT
 LeuGluIleMetThrSerThrTrpValLeuAlaGlyGlyValLeuAlaAlaValAlaAla

1210 1220 1230 1240 1250 1260
 TACTGCCTGGCAACCGGCTGTGTTTCCATCATCGGCCGCTACACCTGAATGATCAAGTG
 TyrCysLeuAlaThrGlyCysValSerIleIleGlyArgLeuHisLeuAsnAspGlnVal

1270 1280 1290 1300 1310 1320
 GTTGTGACTCCTGACAAAGAAATCTTATATGAGGCCTTTGATGAGATGGAAGAATGCGCC
 ValValThrProAspLysGluIleLeuTyrGluAlaPheAspGluMetGluGluCysAla

1330 1340 1350 1360 1370 1380
 TCCAAAGCCGCCCTCATTGAGGAAGGGCAGCGGATGGCGGAGATGCTCAAGTCTAAGATA
 SerLysAlaAlaLeuIleGluGluGlyGlnArgMetAlaGluMetLeuLysSerLysIle

1390 1400 1410 1420
 CAAGCCCTCCTACAACAGGCCACAAGACAGGCCCAAGACATACAGC
 GlnGlyLeuLeuGlnGlnAlaThrArgGlnAlaGlnAspIleGln

Fig. 6a

```

10      20      30      40      50      60
CGCAGACATTCCAAAGTGGCCCATCTGCACGGCTCCCACTGGTAGCGGCAAGACCACTAAGG
  GlnThrPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal

70      80      90      100     110     120
TGCCGGCTGCATATGCGGCCCAAGGTACAAGGTACTCGTCCTGAACCCGTCCGTTGCCG
  ProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla

130     140     150     160     170     180
CCACTTTAGCCTTTGGGGCGTACATGTCTAAGGCACATGGTGTGACCCCTAACATCAGAA
  ThrLeuAlaPheGlyAlaTyrMetSerLysAlaHisGlyValAspProAsnIleArgThr

190     200     210     220     230     240
CTGGGGTGAGGACCATCACCGGGCGGCTCCCATCAGTACTCCACCTATGGTAAGTTCC
  GlyValArgThrIleThrThrGlyAlaProIleThrTyrSerThrTyrGlyLysPheLeu

250     260     270     280     290     300
TTGCCGACGGTGGTTGCTCTGGGGCGCCTATGACATCATATAATATGTGATGAGTGCCACT
  AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSer

310     320     330     340     350     360
CAACTGACTCGACATCCATCTTGGGCATCGGCACAGTCTTGACCAAGCGGAGACGGCTG
  ThrAspSerThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly

```

Fig. 6b

370 380 390 400 410 420
 GAGCGCGGCTCGTCGTGCTCGCTACCGCTACGCCTCCGGGATCGGTCACCGTGCCACATC
 AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro

430 440 450 460 470 480
 CCAATATCGAGGAGGTGGCCCTGTCCACCACTGGAGAGATTCCCTTCTACGGCAAAGCTA
 AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle

490 500 510 520 530 540
 TCCCCATCGAGACAATCAAGGGGGGAGGCATCTCATCTTCTGCCGTTCCAAGAAGAAGT
 ProIleGluThrIleLysGlyGlyArgHisLeuIlePheCysArgSerLysLysLysCys

550 560 570 580 590 600
 GTGACGAGCTCGCTGGAAAGCTGTCAGCCCTCGGAATCAACGCTGTAGCGTACTACCGGG
 AspGluLeuAlaGlyLysLeuSerAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly

610 620 630 640 650 660
 GTCTTGATGTATCCGTCATACCGACCAGCGGAGACGTCGTTGTCGTGGCAACAGACGCTC
 LeuAspValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeu

670 680 690 700 710 720
 TAATGACGGGCTACACCGGTGACTTTGATTTCAGTGATCGACTGCAATACATGTGTACCCC
 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln

730 740 750 760 770 780
 AGACAGTCGACTTCAGCTTGGACCCTACCTTCACCATGAGACGACGACCGTGCCTCAAG
 ThrValAspPheSerLeuAspProThrPheThrIleGluThrThrThrValProGlnAsp

790 800 810 820 830 840
 ACGCGGTGTCACGCTCGCAGCGGCGAGGCAGAACTGGTAGGGGTAGAGGGGGCATATACA
 AlaValSerArgSerGlnArgArgGlyArgThrGlyArgGlyArgGlyGlyIleTyrArg

850
 GGTTTGTGACTCCAG
 PheValThrPro

Fig. 7

10 20 30 40 50 60
 GACGAGCTCGCCGCAAAGCTGTCAGGCCTCGGAGTCAATGCTGTGGCATACTACCGGGGT
 AspGluLeuAlaAlaLysLeuSerGlyLeuGlyValAsnAlaValAlaTyrTyrArgGly

70 80 90 100 110 120
 CTCGATGTGTCTGTCATACCGACGAGCGGGGACGTCGTTGTTGTGGCAACAGACGCTCTA
 LeuAspValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeu

130 140 150 160 170 180
 ATGACGGGCTATACCGGCGACTTTGACTCGGTGATCGACTGCAATACATGTGTCACCCAA
 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln

190 200 210 220 230 240
 ACAGTCGATTTTCAGCTTGGACCCTACTTTTACCATTGAGACGACGACCGTGCCCCAAGAC
 ThrValAspPheSerLeuAspProThrPheThrIleGluThrThrThrValProGlnAsp

250 260 270 280 290 300
 GCGGTGTCGCGCTCGCAGCGGCGAGGCAGGACTGGTAGGGGCAGGGTGGGCATATACAGG
 AlaValSerArgSerGlnArgArgGlyArgThrGlyArgGlyArgValGlyIleTyrArg

310
 TTTGTGACTCCCGAG
 PheValThrProGlu

Fig. 8a

10 20 30 40 50 60
 GTGATGAGCTCGCCGCAAAGCTCTCAAGCCTCGGACTCAACGCTGTAGCATATTACCGGG
 AspGluLeuAlaAlaLysLeuSerSerLeuGlyLeuAsnAlaValAlaTyrTyrArgGly

70 80 90 100 110 120
 GTCTTGATGTGTCGTCATACCGACTAGTGGAGACGTCGTTGTCGTGGCAACAGACGCTC
 LeuAspValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeu

130 140 150 160 170 180
 TAATGACGGGCTATACCGGCGACTTTGACTCAGTGATCGACTGTAACACATGTGTACCC
 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln

190 200 210 220 230 240
 AGACAGTTGATTTGAGCTTGGATCCAACCTTCACCATGAGACGACGACCGTGCCTCAAG
 ThrValAspPheSerLeuAspProThrPheThrIleGluThrThrThrValProGlnAsp

250 260 270 280 290 300
 ACGCGGTGTCGCGCTCGCAGCGGCGAGGTAGGACTGGCAGGGGCGAGGGGCGGCATCTATA
 AlaValSerArgSerGlnArgArgGlyArgThrGlyArgGlyArgGlyGlyIleTyrArg

310 320 330 340 350 360
 GGTGTTGACTCCAGGAGAACGGCCCTCGGGCATGTTGATTCTCGGTCCTGTGTGAGT
 PheValThrProGlyGluArgProSerGlyMetPheAspSerSerValLeuCysGluCys

370 380 390 400 410 420
 GTTATGACGCGGGCTGTGCTTGGTATGAGCTCACGCCGCGAGACCACGGTTAGGTTGC
 TyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArg

430 440 450 460 470 480
 GGGCTTACCTAAATACACCAGGGTTGCCGTCTGCCAGGACCATCTGGAGTTCTGGGAGG
 AlaTyrLeuAsnThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGly

490 500 510 520 530 540
 GCGTCTTCACAGGCCTCACCCACATAGATGCCCATTTCTTGCTCAGACTAAGCAGGCAG
 ValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnAlaGly

Fig. 8b

550 560 570 580 590 600
 GAGACAACCTTTCCCTACCTGGTGGCATACCAAGCTACAGTGTGCGCCAGGGCTCAGGCTC
 AspAsnPheProTyrLeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaPro

610 620 630 640 650 660
 CACCTCCATCGTGGGACCAAATGTGGAAGTGTCTCATACGGCTGAAACCTACGCTGCACG
 ProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGly

670 680 690 700 710 720
 GGCCAACACCCCTGCTGTATAGGCTAGGAGCCGTCCAAAATGAGGTCACCCTCACACACC
 ProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGluValThrLeuThrHisPro

730 740 750 760 770 780
 CCATAACCAAATTCATCATGGCATGCATGTCGGCTGATCTGGAGGTCGTCACCAGCACCT
 IleThrLysPheIleMetAlaCysMetSerAlaAspLeuGluValValThrSerThrTrp

790 800 810 820 830 840
 GGGTGCTGGTGGGCGGAGTCCTTGCAGCTCTGGCCGCATATCGCCTGACAACAGGCAGCG
 ValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrArgLeuThrThrGlySerVal

850 860 870 880 890 900
 TGGTCATCGTGGGTAGGATCATCTTGTCTGGGAGGCCGGCTGTCATTCCCGACAGGGAAG
 ValIleValGlyArgIleIleLeuSerGlyArgProAlaValIleProAspArgGluVal

910
 TCCTTTACCGG
 LeuTyrArg

Fig. 9

10 20 30 40 50 60
 CGACAACCGTGCCCCAAGACGCGGTGTCGCGCTCACAACGGCGGGGTAGGACAGGTAGGG
 ThrThrValProGlnAspAlaValSerArgSerGlnArgArgGlyArgThrGlyArgGly

70 80 90 100 110 120
 GCAGGAGAGGCATCTACAGATTTGTGACTCCGGGAGAACGGCCCTCGGGCATGTTTCGATT
 ArgArgGlyIleTyrArgPheValThrProGlyGluArgProSerGlyMetPheAspSer

130 140 150 160 170 180
 CTTTCGGTCTGTGTGAGTGCTATGACGCGGGCTGCGCTTGGATCGAGCTCACGCCCCCGG
 SerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpIleGluLeuThrProAlaGlu

190 200 210 220 230 240
 AGACCTCAGTTAGGTTGCGGGCTTACCTAAATACACCAGGGTTGCCCGTCTGCCAGGACC
 ThrSerValArgLeuArgAlaTyrLeuAsnThrProGlyLeuProValCysGlnAspHis

250 260 270 280 290 300
 ACCTGGAATTCTGGGAGAGCGTCTTCACAGGCCTCACCCATATAGATGCCCACTTCTTGT
 LeuGluPheTrpGluSerValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSer

310 320 330 340 350 360
 CCCAGACCAAGCAGGCAGGAGACAACCTTCCCCTACCTGGTAGCATACCAAGCTACAGTGT
 GlnThrLysGlnAlaGlyAspAsnPheProTyrLeuValAlaTyrGlnAlaThrValCys

370 380 390 400 410 420
 GCGCCAGGGCCCAGGCTCCACCACCATCGTGGGATCAAATGTGGAAGTGTCTCATACGCC
 AlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeu

430 440 450 460 470 480
 TGAAACCTACGCTACACGGGCCAACACCCCTGTTGTATAGGCTGGGAGCCCTCCAAAATG
 LysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGlu

AGGTCACCC
 ValThr

Fig. 10a

```

1      10      20      30      40      50      60
GTGGTCTCCTGGGTGCCATCGTGGTCAGCCTAACGGGCCGCGACAAGAACCAGGTGCGAGG
  GlyLeuLeuGlyAlaIleValValSerLeuThrGlyArgAspLysAsnGlnValGluG

      70      80      90      100     110
GGGAGGTTTCAGGTGGTCTCCACCGCAACGCAATCTTTCCTGGCGACCTGCGTCAATGGCGT
lyGluValGlnValValSerThrAlaThrGlnSerPheLeuAlaThrCysValAsnGlyVa

      130     140     150     160     170
GTGTTGGACCGTCTACCATGGCGCCGGCTCGAAAACCCTGGCCGGCCCGAAGGGTCCAGTC
lCysTrpThrValTyrHisGlyAlaGlySerLysThrLeuAlaGlyProLysGlyProVal

      190     200     210     220     230
ACCCAAATGTACACTAATGTGGACCAGGACCTCGTCGGCTGGCCGGCGCCCTCCGGGGCGC
ThrGlnMetTyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProSerGlyAlaA

      250     260     270     280     290
GGTCCTTGACACCATGCACCTGCGGCAGCTCGGACCTTTACTTGGTCACGAGGCATGCTGA
rgSerLeuThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAs

      310     320     330     340     350     360
TGTCATTCCGGTGCGCCGGCGGGGCGATAGCAGGGGGAGCCTGCTTTCCCCCAGGCCCTC
pValIleProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProLeu

      370     380     390     400     410     420
TCCTACTTGAAGGGCTCCTCAGGTGGTCCACTGCTTTGCCCTCGGGGCACATTGTGGGCA
SerTyrLeuLysGlySerSerGlyGlyProLeuLeuCysProSerGlyHisIleValGlyI

      430     440     450     460     470     480
TCTTCCGGGCTGCCGTGTGCACCCGGGGGGTTGCGAAGGCGGTGGACTTTGTACCTGTGCA
lePheArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheValProValGl

      490     500     510     520     530     540
GTCTATGGAACTACTATGCGGTCTCCGGTCTTCACGGATAATTCATCCCCCGGCCGTGA
uSerMetGluThrThrMetArgSerProValPheThrAspAsnSerSerProProAlaVal

      550     560     570     580     590     600
CCGCAGACATTCCAAGTGGCCCATCTGCATGCCCCCACTGGCAGCGGCAAGAGCACTAAGG
ProGlnThrPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysV

      610     620     630     640     650     660
TGCCGGCTGCATACGCAGCCAGGGATACAAGGTAAGTCTGTCCTGAACCCGTCCGTTGCCGC
alProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAl

      680     690     700     710     720
CACCTTAGGTTTTGGAGCATATATGTCCAAGGCACATGGTGTGCGACCCTAACATCAGGACT
aThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyValAspProAsnIleArgThr

      740     750     760     770     780
GGGGTAAGGACCATCACTACGGGCGCCCCATTACATACTCCACCTATGGCAAGTTTCTTG
GlyValArgThrIleThrThrGlyAlaProIleThrTyrSerThrTyrGlyLysPheLeuA

```

Fig. 10b

800 810 820 830 840
CCGACGGTGGTTGCTCCGGGGGCGCCTATGACATCATAATATGTGATGAGTGCCACTCAAC
laAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSerTh

860 870 880 890 900
TGA CTGACTTCCATTTTGGGCATTGGCACGGTCCTGGACCAAGCGGAGACGGCTGGAGCG
rAspSerThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGlyAla

920 930 940 950 960 970
CGGCTCGTCGTGCTCGCCACCGCTACGCCTCCAGGATCGGTCACTGTGCCTCATCCCAACA
ArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisProAsnI

980 990 1000 1010 1020 1030
TCGAGGAGGTGGCCTTGTCCAGCACTGGAGAGATTCCCTTCTATGGCAAAGCCATCCCCAT
leGluGluValAlaLeuSerSerThrGlyGluIleProPheTyrGlyLysAlaIleProIle

1040 1050 1060 1070
TGAGACCATCAAGGGGGAAGGCATCTCATTTTCTGCCAC
eGluThrIleLysGlyGlyArgHisLeuIlePheCysHis

Fig. 11

1 10 20 30 40 50 60
GTCGACCCCAATATTAGAACTGGGGTAAGGACCATCACCACGGGCGCTCCCATTACGTAT
ValAspProAsnIleArgThrGlyValArgThrIleThrThrGlyAlaProIleThrTyr

70 80 90 100 110
TCTACCTATGGCAAATTCCTTGCCGACGGTGGTTGCTCTGGGGGCGCCTATGACATCATAA
SerThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleI

130 140 150 160 170
TCTGTGATGAGTGCCACTCAACTGACTCGACTTCCATCTTGGGTATCGGCACAGCCCTGGA
leCysAspGluCysHisSerThrAspSerThrSerIleLeuGlyIleGlyThrAlaLeuAs

190 200 210 220 230
CCAAGCGGAGACGGCTGGAGCACGGCTTGTCGTGCTCGCCACCGCTACGCCTCCAGGGTCC
pGlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrProProGlySer

250 260 270 280
GTCACCGTGCCGCATCCCAACATCGAGGAGGTAGCCTTGCC
ValThrValProHisProAsnIleGluGluValAlaLeu

Fig. 12

1 10 20 30 40 50 60
 GGACAACTCATCTCCCCGGCGGTACCGCAGACATTCCAGGTGGCCCATCTACACGCTCC
 AspAsnSerSerProProAlaValProGlnThrPheGlnValAlaHisLeuHisAlaPr
 70 80 90 100 110
 CACTGGCAGCGGCAAGAGCACTAAGGTGCCGGCTGCATATGCAGCCCAAGGGTACAAAGTA
 oThrGlySerGlyLysSerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLysVal
 130 140 150 160 170
 CTCGTCTGAACCCGTCCGTTGCCGCCACCTTAAGTTTCGGGGCGTATATGTCCAAGGCAC
 LeuValLeuAsnProSerValAlaAlaThrLeuSerPheGlyAlaTyrMetSerLysAlaH
 190 200 210 220 230
 ATGGTGTTGACCCTAATATCAGAACTGGGACAAGGACCATCACCACGGGCGCTCCCATCAC
 isGlyValAspProAsnIleArgThrGlyThrArgThrIleThrThrGlyAlaProIleTh
 250 260 270 280 290
 GTACTCCACCTATGGCAAGTTCCTTGACAGACGGTGGTTGCTCCGGAGGCGCCTATGACATC
 rTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAspIle
 310 320 330 340 350 360
 ATAATATGCGATGAGTGCCACTCAACAGACTCGACTTCCATCTTAGGCATTGGTACGGTCC
 IleIleCysAspGluCysHisSerThrAspSerThrSerIleLeuGlyIleGlyThrValL
 370 380 390 400 410 420
 TGGACCAAGCGGAGACGGCTGGAGCGCGACTCGTCGTGCTCGCCACCGCTACGCCTCCAGG
 euAspGlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrProProGl
 430 440 450 460 470 480
 ATCGGTCACTGTGCCACATCCCAACATCGAGGAGGTGGCCCTGTCCAACACTGGAGAGATT
 ySerValThrValProHisProAsnIleGluGluValAlaLeuSerAsnThrGlyGluIle
 490 500 510 520 530 540
 CCCTTCTATGGCAAAGCCATCCCCATTGAGGCCATCAAGGGGGGGAGGCATCTCATTTTCT
 ProPheTyrGlyLysAlaIleProIleGluAlaIleLysGlyGlyArgHisLeuIlePheC
 550 560 570 580 590 600
 GCCATTCTAAGAAGAAGTGTGATGAGCTCGCCACGAAGCTGTCGGCCCTCGGACTCAATGC
 ysHisSerLysLysLysCysAspGluLeuAlaThrLysLeuSerAlaLeuGlyLeuAsnAl
 610 620 630 640
 TGTAGCGTACTACCGGGGTCTTGATGTGTCCG
 aValAlaTyrTyrArgGlyLeuAspValSer

Fig. 13

1 10 20 30 40 50 60
 CAGGCGAGAGGCCGACAGGGATGTTTGACAGCGTAGTGCTCTGTGAGTGCTATGATGCCG
 GlyGluArgProThrGlyMetPheAspSerValValLeuCysGluCysTyrAspAlaG

 70 80 90 100 110
 GGGCCGCCTGGTACGAGCTTACGCCTGCTGAGACTACGGTGAGACTCCGGGCTTATTTCAA
 lyAlaAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArgAlaTyrPheAs

 130 140 150 160 170
 CACGCCCCGGTTTGCCTGTATGTCAAGACCACCTAGAGTTCTGGGAAGCGGTCTTCACAGGT
 nThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluAlaValPheThrGly

 190 200 210 220 230
 CTCACACACATTGATGCCCCATTCTCTCCAGACGAAGCAAGGAGGAGACAACCTTTGCGT
 LeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnGlyGlyAspAsnPheAlaT

 250 260 270 280 290
 ATCTAACGGCCTACCAGGCCACAGTATGCGCCAGGGCAAAGGCCCCCCTCCTTCGTGGGA
 yrLeuThrAlaTyrGlnAlaThrValCysAlaArgAlaLysAlaProProProSerTrpAs

 310 320 330 340 350 360
 CGTGATGTGGAAGTGTCTAATCAGGCTCAAACCTACATTGACTGGTCCTACCCCCCTCCTG
 pValMetTrpLysCysLeuIleArgLeuLysProThrLeuThrGlyProThrProLeuLeu

 370 380 390 400 410 420
 TACCGCTTGGGTGCCGTGACTAACGAGGTTACCCTGACGCACCCCGTGACGAAATATATCG
 TyrArgLeuGlyAlaValThrAsnGluValThrLeuThrHisProValThrLysTyrIleA

 430
 CCACGT
 laThr

Fig. 14

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1      10      20      30      40      50      60
ATGGGCACGAATCCTAAACCTCAAAGAAAAACCAAAAGAAACACTAACCGTCGCCCACAA
MetGlyThrAsnProLysProGlnArgLysThrLysArgAsnThrAsnArgArgProGln

      70      80      90      100     110
GACGTTAAGTTTCCGGGCGGCGGCCAGATCGTTGGCGGAGTATACTTGTTGCCGCGCAGGG
AspValLysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuProArgArgG

      130     140     150     160     170
GCCCCAGATTGGGTGTGCGCGCGACAAGGAAGACTTCGAAGCGGTCCCAGCCACGTGGGGG
lyProArgLeuGlyValArgAlaThrArgLysThrSerLysArgSerGlnProArgGlyGl

      190     200     210     220     230
GCGCCGGCCCCATCCCTAAAGATCGGCGCTCCACTGGCAAGTCCTGGGGGAAACCAGGATAC
yArgArgProIleProLysAspArgArgSerThrGlyLysSerTrpGlyLysProGlyTyr

      250     260     270     280     290
CCCTGGCCCCCTATATGGGAATGAGGGACTCGGCTGGGCAGGGTGGCTTCTGTCCCCCGAG
ProTrpProLeuTyrGlyAsnGluGlyLeuGlyTrpAlaGlyTrpLeuLeuSerProArgG

      310     320     330     340     350     360
GTTCCCGTCCCTCTTGGGGCCCCACTGACCCCGGCATAGGTTCGCGCAATGTGGGTAAGGT
lySerArgProSerTrpGlyProThrAspProArgHisArgSerArgAsnValGlyLysVa

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CATC
Ile

Fig. 15a

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1      10      20      30      40      50      60
CGCGCAACTTGGGTAAGGTCATCGATACCCTCACATGCGGCTTCGCCGACCTCATGGGGT
  ArgAsnLeuGlyLysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyT

      70      80      90      100     110
ACATTCCGCTTGTCGGCGCCCCCTAGGGGGTGCTGCCAGGGCCCTGGCACATGGTGTCCG
yrIleProLeuValGlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValAr

      130     140     150     160     170
GGTTCTGGAGGACGGCGTGAACATATGCAACAGGGAATTTGCCCGGTTGCTCTTTCTCTATC
gValLeuGluAspGlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIle

      190     200     210     220     230
TTCCTCTTGGCTTTGCTGTCCTGTTTGACCATCCCAGCTTCCGCTTATGAGGTGCGCAACG
PheLeuLeuAlaLeuLeuSerCysLeuThrIleProAlaSerAlaTyrGluValArgAsnV

      250     260     270     280     290
TATCCGGGATATACCATGTACGAACGACTGCTCCAACCTCAAGTATTGTGTATGAGGCAGC
alSerGlyIleTyrHisValThrAsnAspCysSerAsnSerSerIleValTyrGluAlaAl

      310     320     330     340     350     360
GGACATGATCATGCATACCCCCGGGTGCGTGCCCTGCGTTCGGGAGAACAACTCCTCCCGT
aAspMetIleMetHisThrProGlyCysValProCysValArgGluAsnAsnSerSerArg

      370     380     390     400     410     420
TGCTGGGCAGCGCTCACTCCCACGTTAGCGGCCAGGAACACCAGCGTCCCCACTACGACAA
CysTrpAlaAlaLeuThrProThrLeuAlaAlaArgAsnThrSerValProThrThrThrI

      430     440     450     460     470     480
TACGACGGCATGTCGATTTGCTCGTTGGGGCGGCTGCTTTCTGCTCCGCTATGTACGTGGG
leArgArgHisValAspLeuLeuValGlyAlaAlaAlaPheCysSerAlaMetTyrValGl

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Fig. 15 b

490 500 510 520 530 540
 GGATCTCTGTGGATCTGTCTTCCTCGTTTCCCAGCTGTTCACTTTCTCACCTCGTCGGCAT
 yAspLeuCysGlySerValPheLeuValSerGlnLeuPheThrPheSerProArgArgHis

550 560 570 580 590 600
 GAGACAGTACAGGACTGCAACTGCTCAATCTATCCCGGCCACTTGACAGGTCATCGCATGG
 GluThrValGlnAspCysAsnCysSerIleTyrProGlyHisLeuThrGlyHisArgMetA

610 620 630 640 650 660
 CTTGGGATATGATGATGAACTGGTCACCTACAACAGCCCTAGTGGTGTCGCATCTACTCCG
 laTrpAspMetMetMetAsnTrpSerProThrThrAlaLeuValValSerHisLeuLeuAr

680 690 700 710 720
 GATCCCACAAGCTGTCATGGACATGGTGGCGGGGGCTCACTGGGGAGTCCTAGCGGGCCTC
 gIleProGlnAlaValMetAspMetValAlaGlyAlaHisTrpGlyValLeuAlaGlyLeu

740 750 760 770 780
 GCCTACTATTCCATGGTGGGGAAGTGGGCTAAGGTTTTGATTGTGATGCTACTCTTCGCCG
 AlaTyrTyrSerMetValGlyAsnTrpAlaLysValLeuIleValMetLeuLeuPheAlaG

800 810 820 830 840
 GCGTTGACGGGACCACCTATGTGACAGGGGGGACGACAGGCCGCACCACCAGCTCGTTTCGC
 lyValAspGlyThrThrTyrValThrGlyGlyThrThrGlyArgThrThrSerSerPheAl

860 870 880 890 900
 ATCCCTCTTTACACTTGGGTCGCATCAGAAGGTCCAGCTTATAAATACCAATGGCAGCTGG
 aSerLeuPheThrLeuGlySerHisGlnLysValGlnLeuIleAsnThrAsnGlySerTrp

920 930
 CACATCAACAGGACCGCC
 HisIleAsnArgThrAla

Fig. 16

1 10 20 30 40 50 60
 CGCCGGTATGAGACGGCGCAAGACTGCAATTGCTCACTCTATCCCGGTCACGTATCTGGT
 ArgArgTyrGluThrAlaGlnAspCysAsnCysSerLeuTyrProGlyHisValSerGly

 70 80 90 100 110
 CACCGCATGGCTTGGGATATGATGATGAACTGGTCACCTACAACGGCCCTAGTGGTATCGC
 HisArgMetAlaTrpAspMetMetMetAsnTrpSerProThrThrAlaLeuValValSerG

 130 140 150 160 170
 AGCTACTCCGGATCCCACAAGCCGTCGTGGACATGGTGGCGGGGGCCCACTGGGGAGTCCT
 InLeuLeuArgIleProGlnAlaValValAspMetValAlaGlyAlaHisTrpGlyValLe

 190 200 210 220 230
 AGCGGGCCTTGCCCTACTATTCCATGGTGGCGAACTGGGCTAAGGTCTTGGTTGTGATGCTA
 uAlaGlyLeuAlaTyrTyrSerMetValAlaAsnTrpAlaLysValLeuValValMetLeu

 250 260 270 280 290
 CTCTTTGCCGGCGTTGACGACGGGAAGACCACCGTGACGGGGGGGAGCGCAGCCTTCCAGT
 LeuPheAlaGlyValAspAspGlyLysThrThrValThrGlyGlySerAlaAlaPheGlnS

 310 320 330 340 350 360
 CCAGGAAGTTAGTGTCCTTCTTCTACCAGGGCCGAAACAAAATATCCAGCTTGATAACAC
 erArgLysLeuValSerPhePheSerProGlyProLysGlnAsnIleGlnLeuAspAsnTh

 370 380 390 400 410 420
 CAACGGCAGCTGGCACATCAACAGGACTGCCCTGAATTGCAATGACTCCCTCCAAACTGGG
 rAsnGlySerTrpHisIleAsnArgThrAlaLeuAsnCysAsnAspSerLeuGlnThrGly

 430 440 450 460 470 480
 TTCATCGCTGCGCTGTTCTACGCGCACAAAGTTCAATTTCGTCCGGATGCCTAGAGCGCATGG
 PheIleAlaAlaLeuPheTyrAlaHisLysPheAsnSerSerGlyCysLeuGluArgMetA

 490 500 510 520 530 540
 CCAGCTGCCGCCCCATTGACAAGTTCGCGCAGGGGTGGGGTCCCATCACTCACGATACGCC
 laSerCysArgProIleAspLysPheAlaGlnGlyTrpGlyProIleThrHisAspThrPr

 550
 TAAGATCCCGG
 oLysIlePro

Fig. 17

1 10 20 30 40 50 60
GACACCGTATGGCATGGGACATGATGATGAACTGGTCGCCCACGGCTACCATGATTCTGG
HisArgMetAlaTrpAspMetMetMetAsnTrpSerProThrAlaThrMetIleLeuA

70 80 90 100 110
CGTATGTGATGCGCATCCCCGAGGTCGTCATGGACATCATTGGCGGGGCTCACTGGGGCGT
laTyrValMetArgIleProGluValValMetAspIleIleGlyGlyAlaHisTrpGlyVa

130 140 150 160 170
CATGTTGCGCTTGGGCTATTTTCTATGCAGGGGGCTTGGGCAAAAGTCGTTGTCATCCTT
lMetPheGlyLeuGlyTyrPheSerMetGlnGlyAlaTrpAlaLysValValIleLeu

190 200 210 220 230
CTGCTGGCCGCTGGGGTGGATGCGACTACCCTCAGCGTTGGGGGCTCTGCCGCGCACACCA
LeuLeuAlaAlaGlyValAspAlaThrThrLeuSerValGlyGlySerAlaAlaHisThrT

250 260 270
CCGGCGGCCTTGTCGGCTTGTTCAAGCCTGGCG
hrGlyGlyLeuValGlyLeuPheLysProGly

Fig. 18

1 10 20 30 40 50 60
 CGCTTGTCGGCGCCCCCTAGGGGGTGCTGCCAGGGCCCTGGCACATGGTGTCCGGGTTCC
 LeuValGlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValL

70 80 90 100 110
 TGGAGGACGGCGTGAACATGCAACAGGGAATTTGCCCGGTTGCTCTTTCTCTATCTTCCT
 euGluAspGlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLe

130 140 150 160 170
 CTTGGCTTTGCTGTCCTGTTTGACCATCCCAGCTTCCGCTTATGAGGTGCGCAACGTATCC
 uLeuAlaLeuLeuSerCysLeuThrIleProAlaSerAlaTyrGluValArgAsnValSer

190 200 210 220 230
 GGGATATACCATGTACGAACGACTGCTCCAACCTCAAGTATTGTGTATGAGGCAGCGGACA
 GlyIleTyrHisValThrAsnAspCysSerAsnSerSerIleValTyrGluAlaAlaAspM

250 260 270 280 290
 TGATCATGCATACCCCCGGGTGCGTGCCCTGCGTTCCGGGAGAACAACTCCTCCCGTTGCTG
 etIleMetHisThrProGlyCysValProCysValArgGluAsnAsnSerSerArgCysTr

310 320 330 340 350 360
 GGCAGCGCTCACTCCCACGTTAGCGGCCAGGAACACCAGCGTCCCCACTACGACAATACGA
 pAlaAlaLeuThrProThrLeuAlaAlaArgAsnThrSerValProThrThrThrIleArg

370 380 390 400 410 420
 CGGCATGTGCGATTTGCTCGTTGGGGCGGGCTGCTTTCTGCTCCGCTATGTACGTGGGGGATC
 ArgHisValAspLeuLeuValGlyAlaAlaAlaPheCysSerAlaMetTyrValGlyAspL

430 440 450 460 470 480
 TCTGTGGATCTGTCTTCCTCGTTTCCCAGCTGTTCACTTTCTCACCTCGTCGGCATGAGAC
 euCysGlySerValPheLeuValSerGlnLeuPheThrPheSerProArgArgHisGluTh

490 500 510 520 530 540
 AGTACAGGACTGCAACTGCTCAATCTATCCCGGCCACTTGACAGGTCATCGCATGGCTTGG
 rValGlnAspCysAsnCysSerIleTyrProGlyHisLeuThrGlyHisArgMetAlaTrp

550 560 570 580 590 600
 GATATGATGATGAACCTGGTCACCTACAACAGCCCTAGTGGTGTGCGCATCTACTCCGGATCC
 AspMetMetMetAsnTrpSerProThrThrAlaLeuValValSerHisLeuLeuArgIleP

610 620 630 640 650 660
 CACAAGCTGTCATGGACATGGTGGCGGGGGCCCACTGGGGAGTCCTAGCGGGCCTTGCTTA
 roGlnAlaValMetAspMetValAlaGlyAlaHisTrpGlyValLeuAlaGlyLeuAlaTy

680 690 700 710 720
 CTATTCATGGTGGGGAACCTGGGCTAAGGTTTTGATTGTGATGCTACTCTTCGCCGGCGGTT
 rTyrSerMetValGlyAsnTrpAlaLysValLeuIleValMetLeuLeuPheAlaGlyVal

740
 GACGGGACCAC
 AspGlyThr

Fig. 19

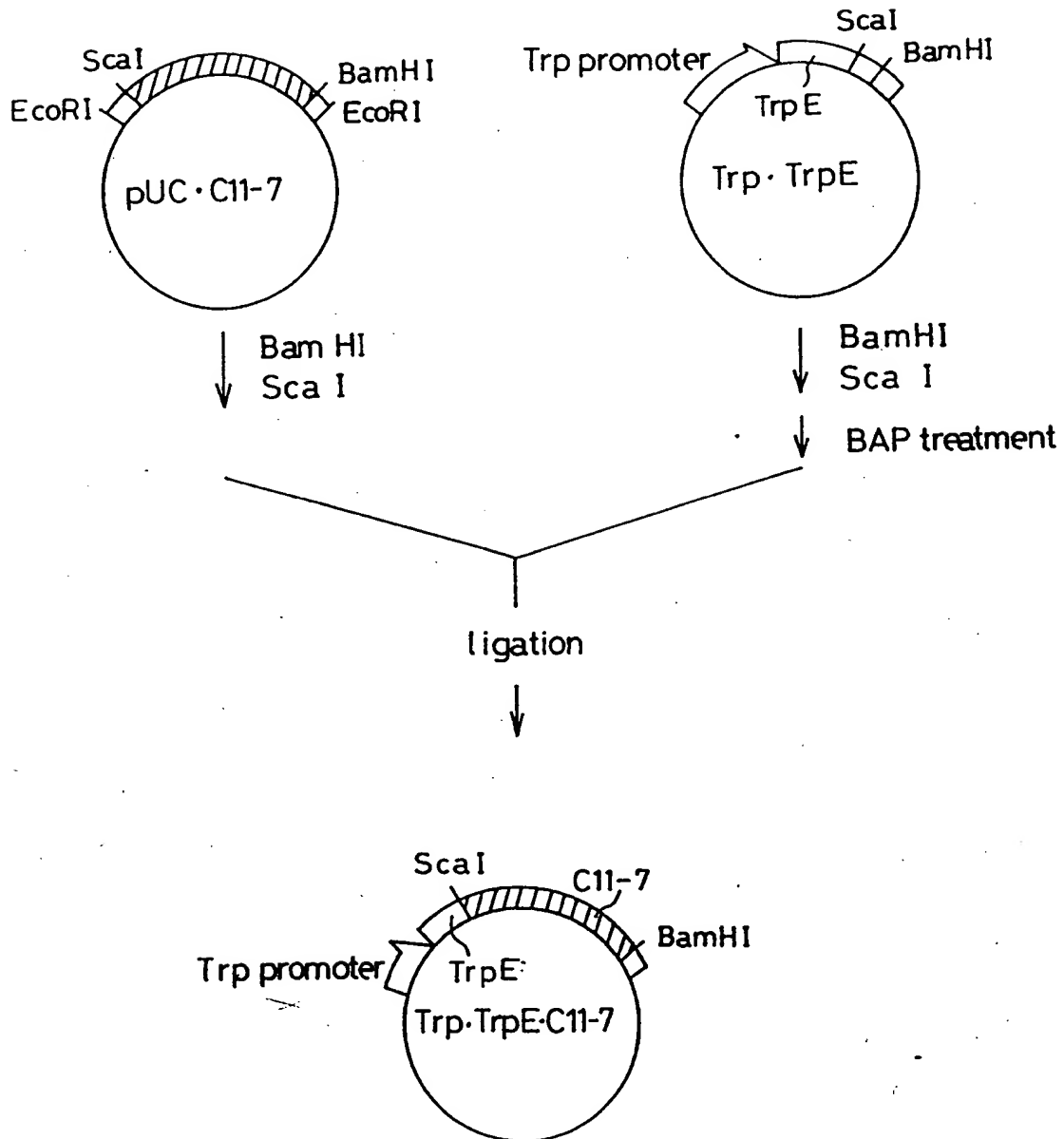


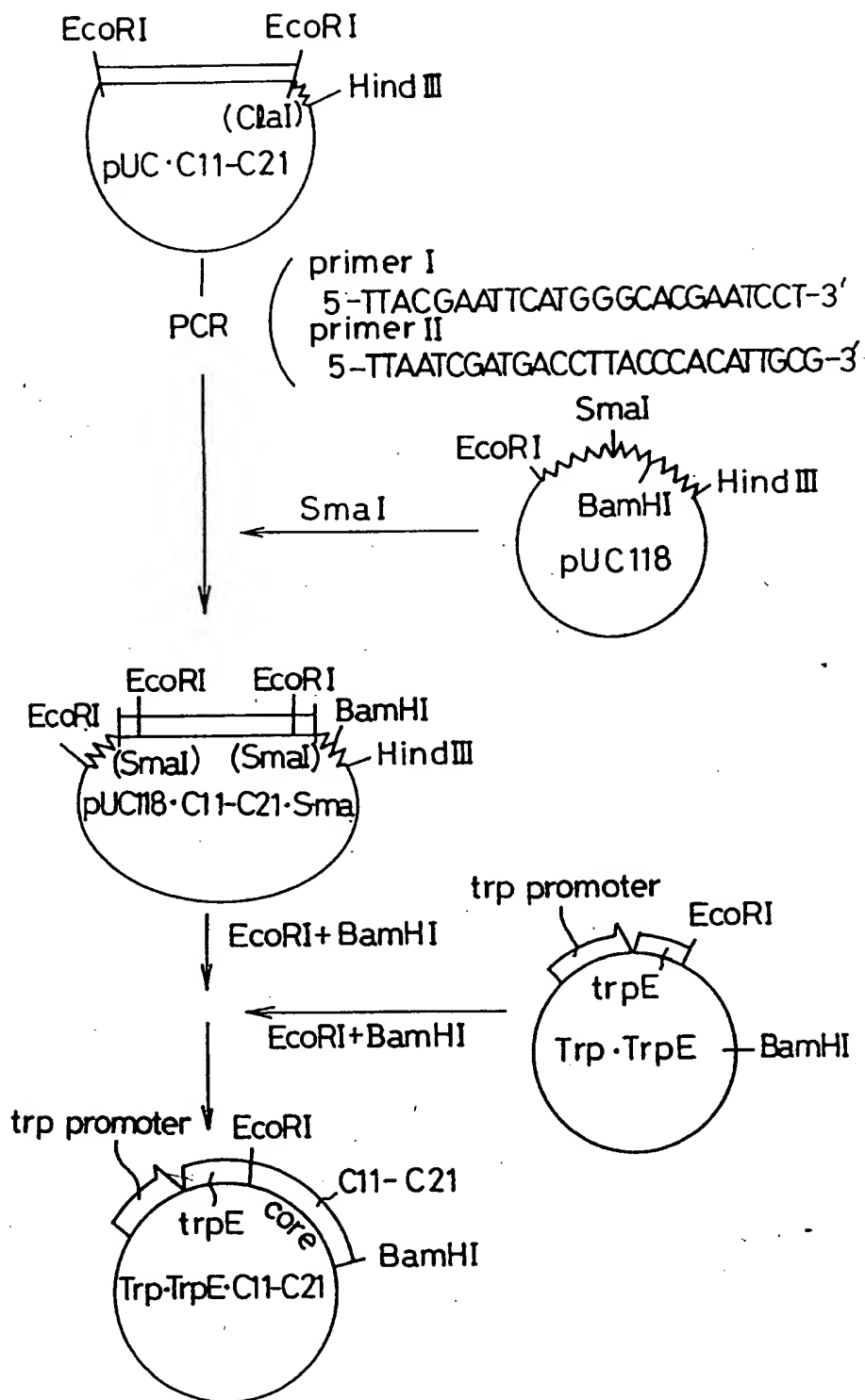
Fig. 20

Fig. 21

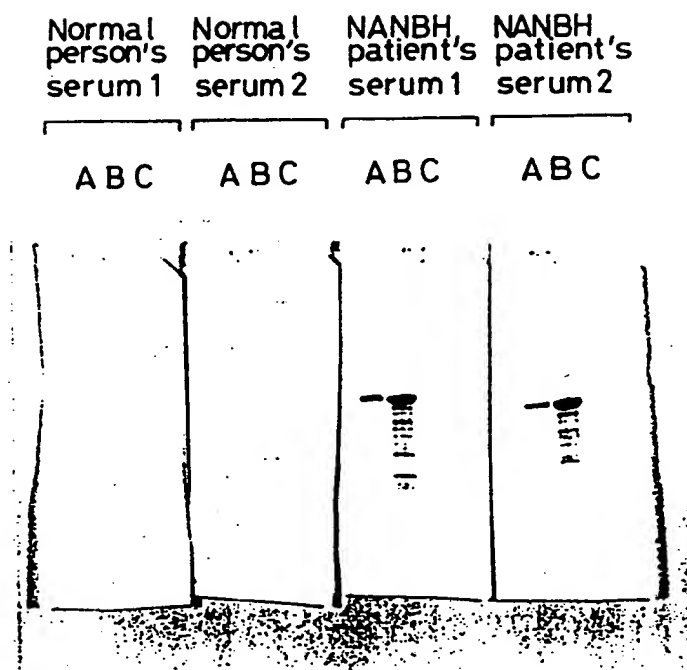


Fig. 22

Normal NANBH
person's patient's
serum serum

AB AB

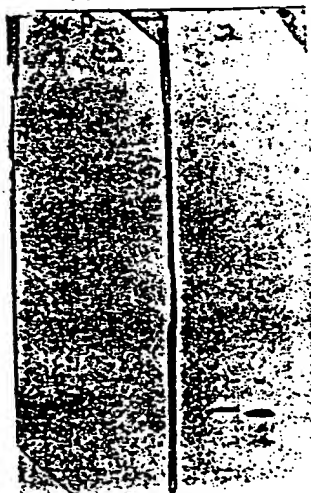
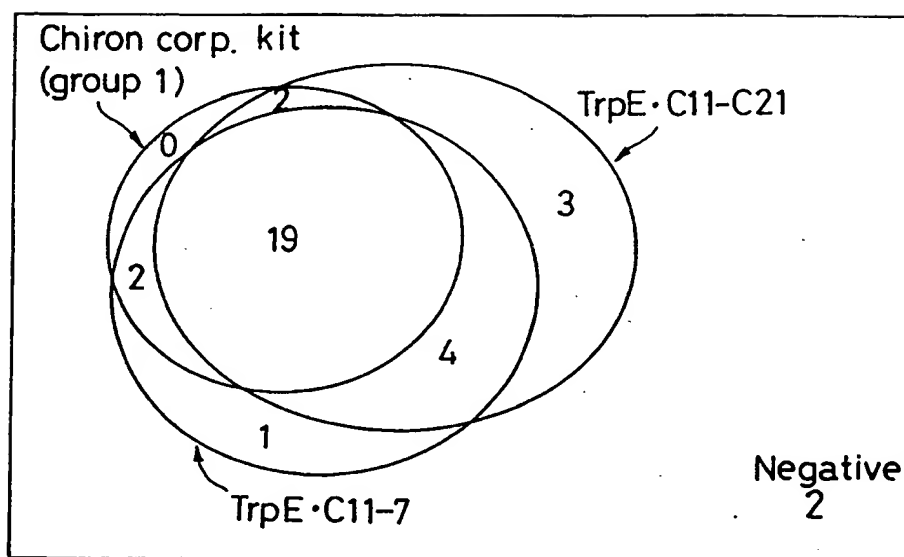


Fig. 23





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⑤④ **Non-A non B hepatitis-specific antigen and its use in hepatitis diagnosis.**

⑤⑦ This invention relates to a DNA fragment encoding a constitutive polypeptide of structural protein in a non-A non-B hepatitis type virus, which is obtained by means of genetic engineering from non-A non-B type hepatitis virus RNA isolated directly from blood plasma of non-A non-B hepatitis patients, to an expression vector containing the DNA fragment, to a transformant obtained by transforming a host with the expression vector and to an expressed polypeptide obtained by culturing the transformant and a process for producing the polypeptide.

Results

The genetic product obtained by expressing the DNA in the transformant is useful as a non-A non-B hepatitis specific antigen for highly accurate diagnosis of non-A non-B hepatitis patients.

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EUROPEAN SEARCH REPORT

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The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 18-10-1991	Examiner JULIA P.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons * : member of the same patent family, corresponding document</p>			

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Application Number

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			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 18-10-1991	Examiner JULIA P.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

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Application Number

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
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The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 18-10-1991	Examiner JULIA P.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>A : member of the same patent family, corresponding document</p>			

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